IN SEARCH OF FREQUENCY DEPENDENT SELECTION

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

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INTRODUCTION AND LITERATURE REVIEW

The theory of evolution has been considered the most important biological generalization in the intellectual history of man. Since the publication, in 1859, of Darwin's Origin of Species a vast amount of evidence on evolution has accumulated. More recently interest has centred on the causes and mechanisms of evolutionary change and attempts have been made to determine the role of various factors involved and their relative importance. The essential prerequisite for evolution is heritable variation, as noted by Darwin himself. Speaking of "the occurrence of profitable variation" he states: "... unless such variation occur natural selection can do nothing". The measurement of such variation and an understanding of the mechanisms through which it is maintained is thus central to evolutionary theory.

The existence of large amounts of genetic variability in natural populations has long been realized and utilized by plant and animal breeders. However it is only in the last decade that direct estimates of variation at the individual gene level have been possible. Before such estimates were made, two conflicting hypotheses existed concerning the genetic nature of populations.

The "classical hypothesis", as upheld by Crow (1961), pictured the usual population as mostly homozygous and genetic variability being maintained by recurrent mutation. Adherents to this view generally based their arguments on considerations of genetic load and believed that polymorphism due to heterozygote superiority would be possible only at a few loci. Dobzhansky and his co-workers (1955)

subscribed to the "balance hypothesis", which pictured the usual population as being on the whole heterozygous, and believed that at the majority of loci genetic variability was maintained by balanced selective forces.

The advent of starch gel electrophoresis made it possible to obtain exact estimates of heterozygosity at enzyme and other protein controlling loci. The first such estimates were reported by Lewontin and Hubby (1966) and Harris (1966). Lewontin and Hubby, working with five natural populations of Drosophila pseudoobscura, estimated that 39% of loci in the genome were polymorphic over the whole species and that on average 12% of loci per individual were heterozygous. Similar estimates of heterozygosity were obtained for humans, by Harris, and since then for mice (Selander and Yang, 1969), snails (Manwell and Baker, 1968) and horseshoe crabs (Selander et al, 1970). From all these studies it appears that the proportion of polymorphic loci in natural populations is far greater than had been previously supposed. This is particularly true when it is borne in mind that results obtained from starch gel electrophoresis will in fact underestimate the number of polymorphic loci present. Only amino acid substitutions which involve a change in the net charge of the protein molecule (i.e. approx. one third) will be detected as electrophoretic variants.

Lewontin and Hubby (1966) admitted to a "dilemma" in trying to explain such a large proportion of polymorphic loci. Heterozygote superiority could not be evoked as a universal explanation because of the resultant load on the population and the difficulty of explaining the adaptive superiority of so many heterozygotes. In

1967 three papers were published which attempted to solve their dilemma. Milkman (1967) pointed out that it was incorrect to calculate fitness as the product of fractional values assigned to each locus, as Lewontin and Hubby did, and that loci could contribute cumulatively. Sved et al.(1967) postulated that fitness rapidly reaches an upper limit because of gene interaction. The maximally fit genotype is sufficiently rare in the population to have little effect in deciding the average selective advantage at individual loci. In this way a large number of polymorphisms can be maintained provided the fitness of the optimum genotype does not greatly exceed that of the population mean. King (1967) presented a similar type of model. He proposed that a form of truncation selection was operating which eliminated that proportion of the population with a fitness score falling below a certain value. However, none of these hypotheses managed to explain the relatively small loss of fitness which has been observed on inbreeding of natural populations.

Previous exponents of the classical hypothesis now proposed the hypothesis of selective neutrality of isoalleles. This was not an entirely new hypothesis as can be seen from the following sentence from Darwin (1859): "Variations neither useful nor injurious would not be affected by natural selection, and would be left either a fluctuating element, as perhaps we see in certain polymorphic species, or would ultimately become fixed." Kimura (1968) pointed to the possible importance of neutral mutations and genetic drift in protein evolution. The uniformity found in the rate of nucleotide substitution in protein molecules during evolution is regarded a strong evidence for the hypothesis. King and Jukes (1969) supported this

theory and proposed that, what they termed "non-Darwinian evolution" was important at the molecular level while the process of natural selection operated at the morphological, functional and behavioural levels. Experimental evidence in favour of the neutralist hypothesis came from studies on esterase-6 in D. melanogaster (McIntyre and Wright, 1966) and esterase-5 in D. pseudoobscura (Yamazaki, 1971). Mukai (1969) found that 10-14 heterozygous loci can explain the genetic variability of viability genes in the second chromosome of D. melanogaster. To reconcile this finding with the estimates of heterozygosity obtained from starch-gel electrophoresis he proposed that most segregating isozyme loci in equilibrium populations involved neutral or nearly neutral isoalleles. The neutralist hypothesis may be adequate to explain the maintenance of large amounts of genetic variation provided a high mutation rate or a large effective population size can be assumed. Recent studies of spontaneous mutation rates at isozyme loci in D. melanogaster (Tobari and Kojima, 1972) have yielded estimates considerably lower than those required by the hypothesis. Kimura (1968) also suggested that migration between populations would mean that high mutation rates and large population size were not essential. However, O'Brien and McIntyre's (1969) study revealed that the Kaduna population of D. melanogaster kept in the laboratory for over 20 years, where presumably migration could not be considered to operate, was as variable as the wild populations analyzed. Studies in this laboratory indicated the contrary, a decrease in variability being found with increased length of time in captivity, though constant environmental conditions, rather than lack of migration, possibly explain such results.

If genetic variation is maintained principally through mutation and random drift then one would expect to find different alternatives fixed in different populations. This situation has not been found. The same allele was shown to be the most frequent in widely separated populations (Prakash, Lewontin and Hubby, 1969). Also rare alleles, which, according to the neutralist hypothesis would be often lost from populations, were found to be present in different populations at similar frequencies. The clinal variation in frequency of some alleles, as found by these authors, also appears to favour the hypothesis that some form of selection is involved. However, Kimura and Ohta (1971) showed how clinal variation and consistency of gene frequencies are not necessarily irreconcilable with the neutralist hypothesis. In fact it appears that any observed pattern of polymorphism can be explained by the neutralist hypothesis simply by assuming appropriate values for N (the effective population size) and m (the migration rate), both of which are, at the present state of knowledge, largely unknown quantities in most situations.

Various other selective mechanisms have been proposed as capable of maintaining genetic variability. Frequency dependent selection, in particular has received much attention since it was proposed by Kojima and Yarbrough (1967) as a "possibe general mechanism responsible for a large amount of the genic polymorphism observed in natural populations." Frequency dependent selection differs from selection favouring heterozygotes in that the selective value of a genotype is not constant but depends in some way on the gene or genotype frequencies.

Wright and Dobzhansky (1946) discussed the possibility of frequency dependent selection in relation to chromosomal polymorphism in D. pseudoobscura. They used the following model of frequency dependent selection, where the heterozygote is always exactly intermediate, and the selective value of each homozygote falls off linearly as the frequency of that allele rises.

Genotype	AA	Aa	aa
Frequency	p^2	2pq	q^2
Selective value (W)	1-a+bq	1	1+a-bq

In this model a and b are positive constants,

the mean fitness, $\bar{W} = 1-(a-bq)(1-2q)$,

the change in gene frequency, $\Delta q = q(1-q)(a-bq)/\bar{W}$

and the equilibrium gene frequency $\hat{q} = a/b$.

They showed how, this model (where the three genotypes are equally fit at equilibrium) and the heterotic model, both fitted their data equally well. Clarke and O'Donald (1964) presented models of frequency dependent selection, with and without dominance, acting alone and in combination with frequency independent selection. In the situation where the heterozygote is distinct and frequency dependent selection is acting alone, the three selective values are given as $1-tp^2$, $1-2tpq$, and $1-tq^2$, where t represents the relation between phenotype and selective value. This model leads to a stable equilibrium where the heterozygote is at a selective disadvantage to both homozygotes.

Wright and Dobzhansky's (1946) model may be considered as gene frequency dependent selection, whereas Clarke and O'Donald's (1964) model is of genotype frequency dependent selection. Clarke (1964) presented models of frequency dependent selection illustrating

how this form of selection may act to modify the dominance of polymorphic genes. More recently Clarke Cockerham et al.(1972) discussed a more general model for frequency dependent selection, which also encompassed the heterotic model.

Kojima and Yarbrough (1967), in proposing frequency dependent selection, considered one of its principle advantages to be the maintenance of variation without envoking large genetic loads. Kojima (1971) compared genetic loads in frequency dependent selection and heterosis. The model of frequency dependent selection was one in which the heterozygote was assigned a fitness of 1 and the fitnesses of the two homozygotes were expressed relative to this. He concluded that segregational load was far less in the case of frequency dependent selection. Kimura and Ohta (1970) claimed that two forms of load exist with frequency dependent selection, a dysmetric load and a drift load. The dysmetric load arises because the equilibrium frequency may not be the frequency giving maximum mean fitness. If the fitness of one homozygote is 1.5 - p and the other two genotypes each have a fitness of 1, then there will be a stable equilibrium, with the three genotypes equally fit, when $p = 0.5$ and $\bar{W} = 1$. If, however, p is less than 0.5 the fitness of that homozygote increases and so also the mean fitness of the population. Kimura and Ohta (1970), using a model of absolute fitnesses, concluded that this dysmetric load could in fact be considerable if a large number of polymorphic loci were maintained in this manner. The drift load arises because in a finite population gene frequency will deviate from equilibrium thus causing a load. However, in a case where the equilibrium frequency is not the frequency of mean maximum fitness, deviations from this

equilibrium will cause an increase in the mean fitness of the population as often as a decrease. A. Robertson (personal communication) has pointed out that a model of frequency dependent selection does not have a definite reference genotype, as the heterotic model does. Thus predictions about load, based on relative fitnesses, as those of Kojima (1971) are perhaps of little value. Valid conclusions can be drawn by using absolute fitnesses, as in Kimura and Ohta (1970). However, what absolute fitnesses one uses is entirely arbitrary and any conclusions may be completely altered by assuming a different set of absolute fitnesses.

Kojima (1971) speculated that the effects of inbreeding would be stronger with heterosis than frequency dependence. Clarke Cockerham et al.(1972) have indicated that the effects of inbreeding may be very complicated in some models of frequency dependent selection and a model with no dominance, they claimed, mimics the heterotic model in almost every respect. Apart from this paper, the expected effects on inbreeding under frequency dependent selection have received little attention in the literature.

Frequency dependent selection has long been thought to play a part in predator-prey relationships, where it can occur between alternate prey species or among the different morphs of a single species. Reighard (1908) working with predatory fish showed that when offered one colour of prey only they became conditioned to that colour and later showed a preference for it when presented with prey of mixed colours. Popham (1941, 1942) studied the influence of the Rudd, Scardinius eryophthalmus, as a predator, in eliminating aquatic insects whose colour was not the same as that of the

environment. In experiments where types of prey offered were not in equal proportions, he found "that the type present in the largest numbers is destroyed relatively faster than the other which is in the minority". He maintained that this was because the choice of the predator varied with the type of prey in view at any one moment. Tinbergen (1960) in studying the density of different prey species in relation to the composition of the diet of the Great Tit put forward the hypothesis of "search images". He found that prey species were at low risk when at low and high densities and at highest risk at moderate densities. He proposed that the low risk at low density resulted because a searching image for a particular prey was only formed by the predator after a number of encounters. The low risk of prey at high densities implies that the searching image is used less above a certain density. Tinbergen explains this by the birds' preference for a mixed diet. Mook et al.(1960) provided further experimental evidence in favour of the searching image hypothesis in their studies of the Great Tit and its prey species the moth, Bapalus piniarius.

Clarke (1962a) extended this concept of searching images. He proposed that it could also be applied to different colour varieties of one species and as such could be involved in the maintenance of polymorphism. He used the term "apostatic selection" to describe selection favouring rare types which stand out from the norm. With increase in frequency a searching image will be formed by the predator. Thus the selective advantage of a particular morph will decrease as its frequency increases and such frequency dependent selection can maintain polymorphism. However, Tinbergen's finding

of low risk at high density does not fit the frequency dependent selection hypothesis. He found that birds concentrate on other prey as soon as one type becomes very common, whereas frequency dependent selection would result in increased predation under such circumstances.

Clarke (1962b, 1969) provided evidence for apostatic selection in the land snails Cepea nemoralis and Cepea hortensis. Allen and Clarke (1968), using artificial baits, found further evidence for apostatic selection being exercised by wild passerines. Owen (1963, 1965) proposed that apostatic selection, in conjunction with density effects, was responsible for the maintenance of polymorphism in populations of the African land snail, Limicolaria martensiana. In all of these studies the ability of the predator to form a search image is assumed. However, Royama (1966, 1970) maintained that Tinbergen's model of search images is not entirely satisfactory despite its popularity. He believed that the assumptions of the model were inappropriate and the logic unconvincing. He has proposed an alternative model to explain his own and Tinbergen's results. Despite this criticism, the theory of search images and apostatic selection has continued to gain support and has recently been extended further to explain polymorphisms in the predator as well as in the prey. Payne (1967) proposed that apostatic selection, and what could perhaps be called avoidance images, which minimize the probability of recognition by the host, were responsible for the variation in colour of parasitic cuckoos. In support of this hypothesis he pointed out that this variation is found in parasitic and not in non-parasitic cuckoos and that it is associated with habitats where the visibility is greatest. Paulson (1973) proposed

the same phenomenon as responsible for polymorphism in diurnal birds of prey.

Batesian mimicry is another example of frequency dependent selection operating in the maintenance of visible polymorphism. The rare mimic has an advantage over the common mimic. The relative frequency of mimics and models is also important, for, as the frequency of mimics increases, relative to models, the probability of the predators' first encounter being with the distasteful model decreases. O'Donald and Pilecki (1970) provided some experimental evidence on the subject. Their results indicate that an extremely distasteful model will provide sufficient protection for both the rare and the common mimic, so that in such cases mimetic polymorphism maintained by frequency dependent selection is unlikely to be found.

In self-sterility alleles in higher plants another form of frequency dependent selection operates. Pollen is only functional on a plant, neither of whose two alleles, at the particular locus involved, is the same as that of the pollen. A rare allele will have an advantage as pollen carrying this allele will be able to pollinate almost every plant in the population.

The first evidence of frequency dependent selection in Drosophila came from work done by Petit (1951, 1958). In her experiments with populations of D. melanogaster, containing mixtures of bar eyed and wild type flies, bar eyed males were found to be less often successful in mating than wild type and their disadvantage became markedly greater as their frequency increased. Frequency dependent mating success was reported by Ehrman et al. (1965) in D. pseudoobscura carrying different chromosomal inversions. This was the first of

many papers on the subject by Ehrman and Spiess and their co-workers (see Spiess and Spiess 1969 and Ehrman 1969 for references). In all cases Elens-Wattiaux observation chambers were used to observe mating behaviour, and it was found that when a particular type of fly was present at a low frequency it mated more often than would be expected. The same phenomenon was observed using strains of flies with different chromosomes, mutants compared with non-mutants, strains from different geographical areas, and flies of the same strain raised at different temperatures. Generally the occurrence of frequency dependent mating success was far more marked in males than in females. It was shown that the mating success of a particular type of male did not depend on whether these males had been raised in the same cultures as their prospective mates or in different cultures. However, Hay (1972) stressed the importance of specific "colony odours". The use of double chambers showed that the mating advantage of the rare kind of males, in the top chamber, disappeared when the bottom chamber contained an excess of this type. Double chambers were constructed such that physical contact between the flies was prevented. From these experiments, it was concluded that physical contact was not important and that the cue by which the females recognize the presence of two types of maleswas airborne and likely to be olfactory. However a later paper (Ehrman, 1970) emphasised the importance of physical contact. Ehrman (1972) reverted to the earlier theory that females appear to obtain information about the frequencies of different types of males by means of airborne olfactory cues. Experiments were undertaken in which crushed flies and various extracts from dead flies were used to test their influence on mating behaviour.

Although the results obtained were not very conclusive Ehrman believed that a lipid or perhaps a steroid was involved. Ehrman *et al.*(1973) reported further progress in isolating and analyzing this pheromone. Other results (Bennet-Clarke and Ewing, 1970 and Petit, 1973) have pointed to the importance of vibrational cues. It seems probable that the mechanism involved is not a simple one and that sound, odour and physical contact all play some role. However, more important than what mediates this frequency dependent behaviour, are the consequences if the phenomenon is at all widespread in natural populations. Such a system of sexual selection could result in the maintenance of balanced polymorphism in the absence of any advantage of the heterozygote.

Petit and Anxolabehere (1968) and Anxolabehere (1971) reported frequency dependent selection operating on larval viability. However, at the level of larval viability, the major impetus for the frequency dependent selection hypothesis has perhaps come from Kojima and his co-workers. Their work extended from studying populations polymorphic for different inversions, as Ehrman and Spiess had done, to studying isozyme variation.

Tobari and Kojima (1967) reported a study of inversion karyotypes in *D. ananassae* in which population cages were initiated with different frequencies of particular inversions and the frequency changes over generations were observed. In all cases the frequencies moved, rapidly in the first few generations, towards an intermediate equilibrium and appeared to stabilize there. The pattern of frequency changes appeared to indicate that the underlying mechanism maintaining the polymorphism was heterozygote superiority. The authors found that a model with constant selective values for each genotype did not satisfactorily explain the results, whereas one in which the selective

values depended on frequency provided a good fit for the data. They concluded that frequency dependent selection alone was responsible for the observed changes in gene frequency. However, a later paper (Kojima and Tobari, 1969a) pointed out that their previous conclusion, was incorrect and that heterozygote superiority was also involved. In the earlier paper fitnesses had been calculated by using zygote frequencies in the population determined by counting at the same stage of development in two successive generations. Prout (1965) pointed out that such a procedure will give incorrect fitness estimates if all selection has not been completed by the time of counting.

Kojima and Yarbrough (1967) reported the finding of frequency-dependent selection operating at the esterase-6 locus in D. melanogaster. Two alleles were segregating at the locus, recognizable through their different electrophoretic mobilities. Their experiments were performed using a population which appeared to be at an equilibrium with the frequency of the faster moving of the two alleles, F, at a frequency of 0.30. Cultures were initiated with different gene frequencies and the genotypes of the emerging adults were observed. A measure of viability fitness was calculated and it was found that the FF genotype had its lowest fitness when the culture was initiated with the F allele at a frequency of 0.70, and its highest when the frequency of F was 0.15. At the point of equilibrium, $q_F = 0.30$, there were no significant differences among the viability estimates of the three genotypes. The fact that $q_F = 0.30$, the point of selective neutrality, was also the point of equilibrium in the original population was

regarded as an indication that viability was the major component of total fitness associated with the esterase-6 locus. Another investigation (Yarbrough and Kojima, 1967) using the same base population, presented further evidence to support the belief that frequency dependent selection was operating at the esterase-6 locus. Eight population cages were set up with the frequency of the F allele at either 0.9 or 0.1. The change in gene frequency over generations was observed. All, but one, of the eight cages converged to gene frequencies between 0.25 and 0.50. Again an effort to fit constant selective values to the three genotypes failed but a frequency dependent model adequately explained the results. In the graphs presented, of the changes of selective fitnesses over time in the seven cages which did converge, five out of seven showed the heterozygote superior at some stage and in one the heterozygote had a superior fitness throughout. No mention of this was made in the text and their conclusion that frequency dependent selection alone is responsible for the maintenance of the polymorphism might be questioned.

Huang et al.(1971) proposed a possible mechanism through which frequency dependent selection may operate. Media preconditioned by either FF, FS or SS esterase-6 genotypes was obtained and the subsequent survival from egg to adult of each of the three genotypes was measured in each of the three conditioned media. The results reported were very clear cut and showed that the viability of the FF genotype was lowest in media conditioned by FF and highest in media conditioned by SS. Similar results were reported for the other homozygote and the heterozygote. The authors proposed that differential utilization of

media was the means through which frequency dependent selection operated at the egg to adult viability level. Each genotype is envisaged as occupying a separate ecological niche in the medium and utilizing particular nutrients required by that genotype and thereby reducing the chance of survival for others of its own genotype that follow. These experiments were extended by Kojima and Huang (1972). Six different density levels were used and the importance of population density in the establishment of frequency dependent selection was stressed. The authors pointed out that frequency dependent selection cannot be expected to operate when population density is low. However, what exactly they considered as a low density is unclear. The density used in the earlier paper (Huang et al., 1971) here yielded survival percentages which the authors considered as possibly too low to demonstrate frequency dependent selection. The table of larval viabilities presented in this second paper includes a somewhat misleading standard error. Five replicates of each media x genotype combination were set up and the results are presented as the mean survival over the five replicates with a standard error. However the standard error is simply the standard error of a proportion and as such gives no indication of the variation between replicates.

A study of alcohol dehydrogenase, also segregating for two alleles, (F and S), revealed frequency dependent selection again operating at egg to adult viability (Kojima and Tobari, 1969b). The experiments were performed with one of four replicate cages which was at an equilibrium with $q_F = 0.60$. Two of the other three cages were fixed for the F allele and in the third $q_F = 0.90$. Such differences in gene frequency would appear to indicate the absence of

strong selective forces operating at the locus. However the experimental results indicated otherwise and the authors reported a "substantial degree of viability selection existing in the egg-to-adult interval". Some explanation for these apparently contradictory observations would appear necessary.

The possibility of linkage playing a role in some of Kojima's work should perhaps be considered, particularly in the case of esterase-6 where the population used originated from two inbred lines. Yamazaki (1971) considered his population to be in linkage equilibrium after 15 years of random mating while Kojima and Yarbrough (1967) considered 30 generation adequate to satisfy this condition. It is possible that the effect of blocks of genes rather than single loci were being studied and perhaps the esterase-6 and alcohol dehydrogenase loci should be regarded as indicators of the mode of selection rather than the cause. McIntyre and Wright (1966) considered the genetic background to be of major importance in cage experiments similar to those of Kojima. They found that if the esterase-6 alleles were separated from interacting gene complexes they could be neutral or nearly neutral in selective value. In cases where no effort was made to control the genetic backgrounds of the founders a return to equilibrium was rapid and this they concluded to be a by-product of selection for linked interacting gene complexes.

Notwithstanding these possible objections the evidence amassed by Kojima and his co-workers on frequency dependent selection operating on larval survival, at the esterase-6 locus at least, is considerable. However the generality of the mechanism requires further investigation. Secondly frequency dependent mating, which

appears from Ehrman and Spiess' work to be such a widespread phenomenon, has not been investigated for isozyme loci. Also evidence of frequency dependent selection, either during larval viability or mating, has not as yet been reported for a quantitative trait. It was with these three points in mind that the initial work reported here was conceived, the aims of which were to investigate, in Drosophila melanogaster, the possible occurrence of frequency dependent selection (a) through viability and (b) through mating at (i) esterase-6

(ii) alcohol dehydrogenase

- or the areas of chromosome marked by these loci.

and at

(iii) loci responsible for the maintenance of genetic variation in sternopleural bristle number.

The search for frequency dependent selection was later extended to a more general level, and again both larval viability and mating were investigated.

CHAPTER 2

FREQUENCY DEPENDENT SELECTION AT SPECIFIC LOCI

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I. INTRODUCTION

Frequency dependent selection has been proposed as being responsible for the maintenance of genetic variation at the alcohol dehydrogenase (ADH) and esterase-6 (Est-6) loci in *Drosophila melanogaster* (Kojima and Tobari, 1969b and Kojima and Yarbrough, 1967). The actual mechanisms involved in the maintenance of genetic variation for sternopleural bristle number have been the subject of some controversy. Kearsey and Barnes (1970) and Linney *et al.*(1971) believe that stabilizing selection with an intermediate optimum is responsible for maintaining variability. They propose that flies with extreme bristle scores are less fit simply because of their phenotype for bristles. However, the selection they observe in their experiments is during the larval stage and cannot therefore be selection for bristle phenotype. Robertson (1955 and 1963) has pointed out the assumptions implied in treating the observed relationship between bristle score and fitness in this manner. Such a causal relationship implies that genes affecting sternopleural bristle number affect no other character and have their effect on fitness only through bristle number; a "simple, but biologically unreal" model. Another pitfall of the optimum model is that it will not in fact maintain genetic variability but will result in fixation (Robertson, 1963). The alternative heterozygous model maintains that the decrease in fitness of extreme phenotypes is a consequence of increased homozygosity. This model will result in the maintenance of genetic variability through overdominance for

fitness. Robertson (1970) refers to experimental evidence which indicates that bristle number is not subject to strong natural selection because of any direct effect on fitness but is perhaps a character of trivial importance. Spiers (1974) also has found little evidence of stabilizing selection operating on bristle number. However frequency dependent selection has not previously been directly investigated in connection with sternopleural bristle score or any other metric character.

Frequency dependent selection operates through advantage of the rare allele thus causing it to increase in frequency to a certain equilibrium level. At frequencies above this level the allele is selected against causing a return to the equilibrium. The selective value of any particular allele therefore is not a constant but alters with gene frequency. With heterozygote superiority - the most commonly proposed mechanism for the maintenance of polymorphism - the selective value of an allele is constant and variation is maintained through selection favouring heterozygotes. Many of the previous investigations of frequency dependent selection in segregating populations have involved perturbation type experiments where cages were set up at extreme frequencies and the return to equilibrium was observed. In such experiments both selection of heterozygotes and selection of the rare allele will result in a similar return to the equilibrium gene frequency. Deciding on which mode of selection is actually operating is difficult (Tobari and Kojima, 1967, Kojima and Tobari, 1969a). The design of the experiments to be reported here is such as to overcome this difficulty and make it possible to

distinguish frequency dependent selection from other possible mechanisms. The equilibrium gene frequencies, at ADH, Est-6 and sternopleural bristle loci, in the population of Drosophila melanogaster studied are not altered but the different alleles within the segregating population in turn become rare, as described later, through competing the segregating population with two different monomorphic lines. These lines are fixed for the opposite alternatives at the loci under observation. If rarity confers an advantage, alleles, not present in the competing monomorphic line, would be expected to increase in frequency in the segregating population. In other words, frequency dependent selection will drive the gene frequencies in the segregating population in the opposite direction from those in the monomorphic competitor. If on the other hand the equilibrium is maintained through heterozygote superiority, the selective value of an allele will be constant and the presence of an excess of one allele in the competing line will not effect the constant selective values and no change in gene frequency would be expected. Selective neutrality would also result in no change in gene frequency under these conditions.

II. MATERIALS AND METHODS

(i) Culture Conditions

Flies were cultured in $\frac{1}{2}$pint bottles containing a standard cornmeal molasses medium and were maintained at 25°C.

(ii) Stocks

The segregating population, used in the three experiments to be reported here, was the standard Kaduna population, previously described by Clayton *et al.* (1957). This population has been maintained in the laboratory under constant conditions since 1949 and has a population size of approximately 5,000. It is segregating for two electrophoretically distinguishable alleles at both ADH and Est-6. At the ADH locus the faster moving of the two alleles (F) is at an equilibrium frequency of around 0.60. At the Est-6 locus the slower moving allele (S) is the most frequent, being at an equilibrium frequency of around 0.70. The population has an intermediate sternopleural bristle score of about 18 bristles and is assumed to be segregating for alleles tending to increase bristle score, designated H for high, and alleles tending to decrease bristle score, designated L for low.

The two competitor lines, C_3A and DF, had been selected from the standard Kaduna population. The C_3A line was produced by selection for high sternopleural bristle number (da Silva, 1961), and it has a mean bristle score of about 49. It is therefore assumed that at the bristle loci the H alleles have been fixed. This line is also fixed for the F allele at both ADH and Est-6. Thus when the segregating population is in cultures with C_3A, the L bristle alleles, the S ADH alleles and the S Est-6 alleles in the segregating pop-

ulation will be rare. The DF line is the D line of Osman and Robertson (1968), produced by selection for low sternopleural bristle number. It has a mean bristle score of 8, the L alleles being fixed, and the S allele is fixed at both enzyme loci. In cultures with DF the H bristle alleles and the F enzyme alleles in the segregating population will be rare. The C_3A and DF lines used here were obtained from Professor A. Robertson and were both homozygous for the eye colour gene claret (ca) located on the third chromosome. In the second experiment it was also necessary to have visible markers in Kaduna. Two fourth chromosome markers, cubitus interuptus (ci) and eyeless Russian (ey^R) were introduced into a sample of the Kaduna population for this experiment.

(iii) Electrophoretic Techniques

Horizontal starch gel electrophoresis was used for both enzymes. A continuous Tris EDTA borate system with pH 8.0 was found to be most satisfactory. The buffer in the electrode tanks was 0.5M while a 0.05M solution of the same buffer was used in the gels. Gels were made with 1 gm Connaught starch to 10ml buffer. 1 mg/ml magnesium chloride was added to the gels just before pouring to improve the resolution of the Est-6 bands. Flies were ground individually in a drop (approximately 0.02ml) of distilled water and the homogenate was then absorbed into 5 x 5 mm cellulose acetate paper which was inserted into a slit in the gel. On this system it was possible to run two rows, each containing 24 inserts, on the one gel. The electrophoresis was performed at $0^{\circ}C$ (an icebag being placed on top of the gel for this purpose) at a voltage gradient of 17 - 20 volts/cm for $1\frac{1}{2}$ hours. After the completion

of the electrophoresis the gels were sliced horizontally into two, one slice being stained for ADH and the other for Est-6. The stain used for ADH was a modification of the method of Shaw and Koen (1965) and consisted of

15 mg	NAD
15 mg	NBT
2 mg	PMS
5 ml	isopropanol
100 ml	0.1 M Tris-H.Cl pH 8.5

The Est-6 stain was a modification of that used by Wright (1963) consisting of

25 mg	αnaphthyl acetate dissolved in 1 ml acetone
75 mg	Fast Blue BB salt
100 ml	0.067 M phosphate buffer pH 6.4

Both stains were allowed to develop at room temperature.

(iv) Experimental Design

(a) Experiment 1

In this experiment the competition between Kaduna and the competitor stock took place from egg laying to emergence of the progeny. Cultures were initiated with three to four day old Kaduna females, which had been previously mated to Kaduna males, and competitor females, mated to males from the same line. Both competitor lines were homozygous for ca and the progeny were thus distinguishable from the Kaduna progeny which carried no markers.

Each replicate bottle was set up with a total of 200 mated females, 160 competitor and 40 Kaduna, which were allowed to lay eggs over a 16 hour period and then discarded. It was necessary to avoid excessive overcrowding because in such cases the Kaduna flies, being fitter than either of the competitors, emerged in large numbers while few competitors survived the overcrowding. The emerging Kaduna progeny were collected as virgins. A sample of 40 males and 40 females was taken and aged together over a three day period. The mated females were then used to set up the next generation. Mated competitor females, for the next generation, were obtained from separate cultures as on occasions insufficient emerged from the experimental bottles. The same procedure was carried out for 5 generations. One sample of Kaduna progeny from the 5th generation was typed for ADH and Est-6, and another was scored for sternopleural bristle number. The experiment consisted of 10 replicates in all, 5 with caC_3A as the competitor and 5 with caDF.

(b) Experiment 2

This experiment was also run over 5 generations but in this case competition between Kaduna and the competitor took place from mating of parents through to emergence of progeny. Cultures were set up with three to four day old virgin females and males from Kaduna and the competitor line. In order to be able to distinguish progeny from the three possible matings, visible markers were used. These were $ciey^{R}K$, caC_3A and caDF as previously described. Again a ratio of 4 competitor to one Kaduna was used with a total of 200 pairs per replicate bottle. Mating and egg laying took place over

24 hours after which time the flies were discarded. Emerging progeny were collected as virgins but only those resulting from matings between ciey^{R}K flies were kept, all others being discarded. The next generation was set up with a sample of 40 pairs of these ciey^{R}K progeny and 160 pairs of the competitor line, again obtained from separate cultures. The ciey^{R}K progeny of the 5th generation were analyzed for the three loci being studied. This experiment consisted of 8 replicates, 4 with each competitor.

(c) Experiment 3

Here the segregating population was not in direct competition with the monomorphic lines but was cultured in media preconditioned by them. The technique used for conditioning media was that described by Huang et al. (1971). Two types of conditioned media were obtained, one conditioned with C_3A and the other with DF. A specific number of first instar larvae of the conditioning line was transferred to 5cc of cornmeal molasses medium in a vial. The vials were maintained at 25^{o}C until most of the larvae had pupated, at which stage they were removed from the vials which were subsequently frozen to kill any remaining larvae. When the vials had returned to room temperature first instar larvae from the Kaduna population were transferred into each vial. The experiment consisted of two different larval densities with six replicates for each type of conditioned medium, giving a total of 24 vials in all. The lower density, density 1, consisted of 75 first instar larvae of the conditioning line followed by 150 first instar larvae of the Kaduna population. The higher density, density 2, consisted of double these numbers ie. 150 of the conditioning line

followed by 300 of the Kaduna. Collection of emerging adults was continued until all the flies had emerged. A sample from each vial was then typed for ADH and Est-6 and another sample was scored sternopleural bristle number.

III RESULTS

The results are presented in Tables 2.1 to 2.9 and in Figures 2.1 to 2.3. For each table, of gene frequencies or sternopleural bristle scores, there is a corresponding figure, where the same results are presented in histogram form.

The results for the two enzyme loci are presented as the frequency of the faster moving of the two alleles, q_F. This was estimated, for each replicate, from a sample of 48 flies, 24 males and 24 females. As there were no consistent differences between the gene frequency in males and in females, a combined estimate was used. The standard error of the gene frequency in each replicate was calculated as $\frac{pq}{2n}$, where $2n = 96$, the number of genes sampled. The value of n however varied slightly because on some gels the genotype of all 48 flies could not be ascertained accurately and was therefore omitted.

In the tables of enzyme results, the observed number of each of the three genotypes is presented with the Hardy Weinberg expectation. A goodness of fit test, of observed and expected numbers was performed, except in cases where the expected number was less than five. The value of chi square with one degree of freedom is presented in the tables. The number of degrees of freedom of the chi square is the number of classes of observations, minus one, minus the number of parameters estimated from the data. In the results presented, the data themselves were used to estimate the gene frequency on which the expectations are based and therefore the chi square has one degree of freedom. Out of a total of 52 χ^2 tests presented, four are significant (one at the 5% level, two at the $2\frac{1}{2}$% level and

one at the 1% level). This is slightly more than would be expected by chance alone. However, as these significant chi squares do not point to any consistent departures from Hardy Weinberg, it appears reasonable to attribute them to sampling.

In the analysis of variance presented with each table of gene frequencies, for the first two experiments, the expected values for the three mean squares calculated are as follows -

Source	E(MS)
Between competitors	$\frac{\sigma^2_w}{N} + \sigma^2_b + 5\sigma^2_c$
Between replicates, within competitors	$\frac{\sigma^2_w}{N} + \sigma^2_b$
Within replicates	$\frac{\sigma^2_w}{N}$

where σ^2_w is the component of variance within replicates, σ^2_b the component of variance between replicates, σ^2_c the component of variance between competitors and N the total number of replicates. The within replicates mean square is the binomial error, calculated as $\frac{\bar{p}(1-\bar{p})}{2n}$, where 2n = 96, the number of genes samples per replicate. This mean square has an infinite number of degrees of freedom, but it can only be used to test for the main effect, of differences between competitors, if the replicates are homogeneous, i.e. if σ^2_b is zero.

(i) Experiment 1

(a) ADH

Table 2.1 and Figure 2.1a give the gene frequencies in Kaduna at ADH, after five generations of competition with DF (D replicates)

Table 2.1

(a) Observed and expected genotype numbers, chi square and gene frequency at ADH in Kaduna after five generations competition, during the egg to adult stage, with DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.57 ± 0.03.

(b) Analysis of variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	28	29	18	17	2	3	-	0.77±0.04
2	11	10	21	24	16	15	0.54	0.45±0.05
3	12	14	27	24	9	11	1.02	0.53±0.05
4	23	23	21	20	4	4	0.05	0.70±0.05
5	11	12	25	24	12	13	0.02	0.49±0.05
							$\bar{q}_F$ =	0.59±0.06

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2	q_F
1	28	29	19	16	1	2	-	0.79±0.04
2	20	18	19	22	9	7	1.20	0.61±0.05
3	11	13	27	24	10	12	1.02	0.51±0.05
4	16	16	23	23	9	9	0.00	0.57±0.05
5	12	12	24	24	12	12	0.00	0.50±0.05
							$\bar{q}_F$=	0.60±0.05

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.00016	<1.0	
Between replicates Within competitors	8	0.0166	6.64	< 0.001
Within replicates		0.0025		

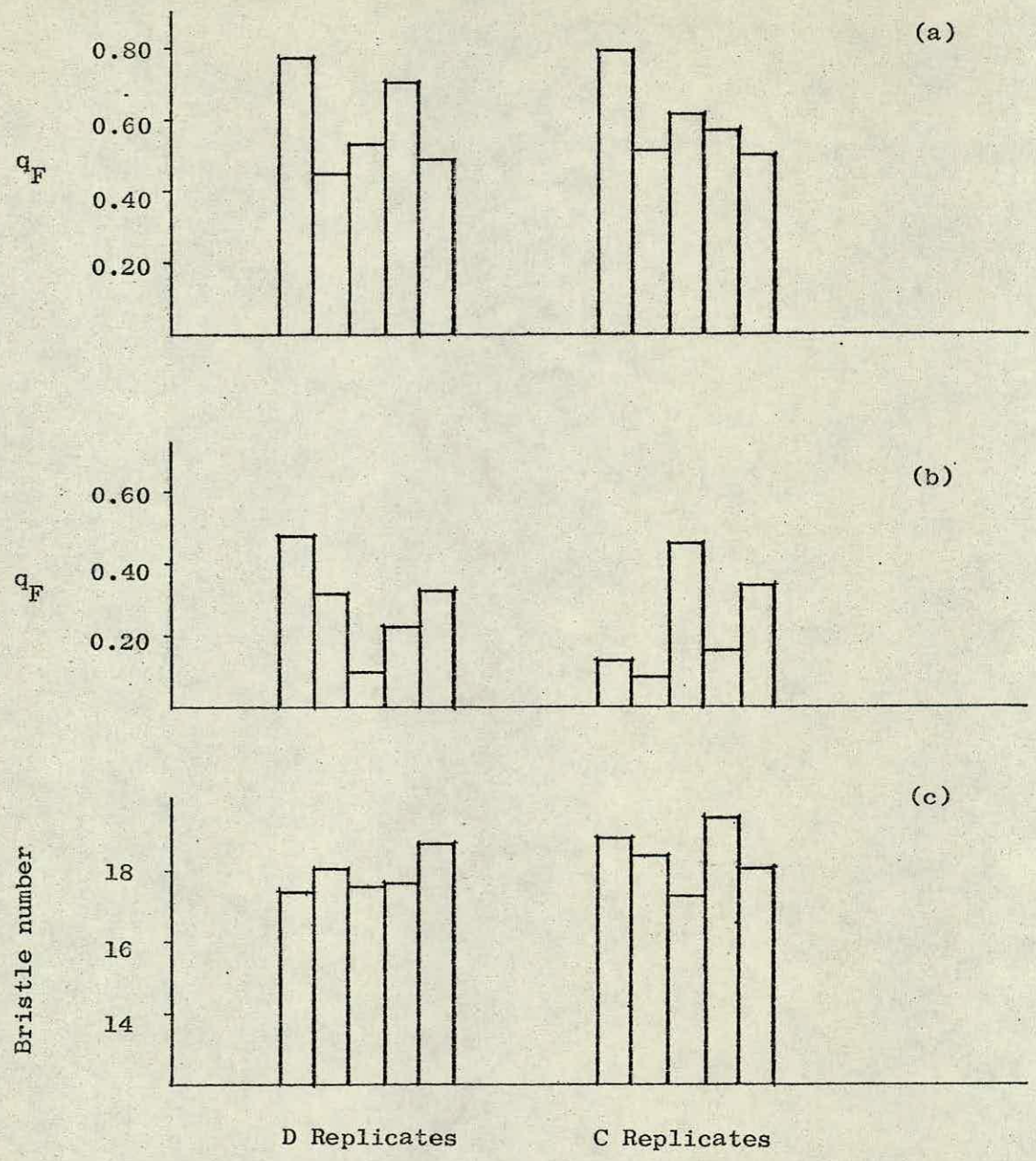

Fig. 2.1. Experiment 1. Gene frequency at ADH(a) and EST-6(b) and sternopleural bristle number (c).

and C_3A (C replicates). In cultures with DF the fast allele in Kaduna is rare as DF is fixed for the slow allele. In cultures with C_3A the fast allele is common as C_3A is fixed for fast. Frequency dependent selection would be expected to cause an increase in the frequency of fast (q_F) in the D replicates and a decrease in q_F in the C replicates. From Table 2.1 it is clear that this has not occurred. Two of the five D replicates have increased in the frequency of fast but the other three have decreased. Over the five replicates the $\bar{q}_F$ is 0.59 ± 0.06, which is not significantly different from the initial q_F of 0.57. Two of the five C replicates have increased in q_F and three have shown a decrease. The overall $\bar{q}_F$ of 0.60 ± 0.05 again is not significantly different from the initial q_F. There is clearly no difference between the overall means of the two sets of replicates, a F_8^1 value of less than 1.0 being obtained in the analysis of variance when difference between competitors is tested. The component of variance between replicates is significant. When the mean square between replicates is compared with the binomial error a F_∞^8 value of 6.44 ($p < 0.001$) is obtained. This would be expected as a result of the accumulation of drift variance over the five generations during which the experiment was run.

(b) Est-6

Table 2.2 and Figure 2.1b present the gene frequencies at Est-6. Here again frequency dependent selection would be expected to result in increased values of q_F in the D replicates and decreased values in the C replicates (DF being fixed for the slow allele and C_3A for the fast allele). No consistent pattern is

Table 2.2

(a) Observed and expected genotype numbers, chi square and gene frequency at Est-6 in Kaduna after 5 generations competition, during the egg to adult stage, with DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.30 ± 0.03.

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	9	10	23	21	11	12	0.37	0.48±0.05
2	4	4	19	18	19	19	-	0.32±0.05
3	0	0	9	8	37	37	-	0.10±0.03
4	4	2	13	16	28	26	-	0.23±0.04
5	4	5	24	21	20	21	0.68	0.33±0.05
								$\bar{q}_F$ = 0.29±0.06

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	0	1	12	11	36	36	-	0.13±0.03
2	0	0	9	8	39	39	-	0.09±0.03
3	12	10	20	24	16	14	1.35	0.46±0.05
4	0	1	15	13	33	34	-	0.16±0.04
5	8	6	17	22	23	21	2.00	0.34±0.05
								$\bar{q}_F$ = 0.24±0.07

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.0078	< 1.0	
Between replicates Within competitors	8	0.0222	10.57	<0.001
Within replicates		0.0021		

apparent in these results. Three of the D replicates show an increase in q_F from the initial q_F of 0.30 and two show a decrease, while two of the C replicates show an increase in q_F and three a decrease. The overall gene frequency in both is lower than the initial gene frequency, being 0.29 $\pm$ 0.06 for D and 0.24 $\pm$ 0.07 for C, but these decreases are not significant. The overall difference between the D and C replicates of 0.05 is in the direction expected with frequency dependent selection but it is not significant. The F^1_8 value for differences between competitors given in the analysis of variance is less than 1.0. Here also a significant difference between replicates is apparent ($F^8_\infty \pm 10.57$; $p < 0.001$).

(c) Sternopleural bristles

Table 2.3 gives the mean and standard error of sternopleural bristle score in each replicate. Figure 2.1c presents the same results in histogram form. The mean for each replicate was estimated from a sample of 40 flies, 20 males and 20 females. A hierarchical analysis of variance, with two competitors, five replicates per competitor and 40 observations per replicate, is also presented in Table 2.3. DF being the low bristle line and C_3A the high bristle line, frequency dependent selection would be expected to increase bristle score in the D replicates and decrease it in the C replicates. This has not occurred, in fact the D replicates have an overall mean of 17.91, which is lower than that of the C replicates at 18.45. This difference is not significant ($F^1_8 = 1.55$; $p > 0.10$). As with the enzyme results no consistent pattern within a competitor is apparent, some replicates increase in bristle score while others decrease. Again there is a significant difference between replicates, within competitors, ($F^8_{390} = 4.75$; $p < 0.001$).

Table 2.3

(a) Means and standard errors of sternopleural bristle score in Kaduna after five generations competition, during the egg to adult stage, with DF (D Replicates) and C_3A (C Replicates).

(b) Analysis of Variance.

(a)	D Replicates	C Replicates
Replicate No.		
1	17.48±0.25	18.95±0.44
2	18.05±0.30	18.43±0.36
3	17.58±0.27	17.35±0.21
4	17.65±0.33	19.48±0.36
5	18.80±0.30	18.08±0.30
Overall Mean	17.91±0.24	18.45±0.36

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	29.70	1.55	> 0.10
Between replicates Within competitors	8	19.16	4.75	< 0.001
Within replicates	390	4.04		

The possibility of contamination playing a role in this experiment cannot be entirely ruled out. No visible markers were used in the Kaduna population. Consequently if any of the newly emerged Kaduna females had mated with competitor males, before removal from the cultures, this could not have been detected. Such an introduction of genes from the competitor line would serve to counteract the effect of frequency dependent selection, which will drive gene frequency in the segregating population in the opposite direction from that of the competitor. Closer observation of the results for sternopleural bristles can be of help in deciding if such contamination has occurred to any significant extent. Inclusion of progeny from matings between Kaduna and the competitor, as well as giving rise to alterations in bristle score, will result in increased variation within replicates. The three highest C replicates have also the largest within replicate variance. On examining individual fly scores, it was found that in replicate 1, where the variance is highest of all, there were two flies with 26 bristles. In all the other replicates, of the three experiments reported here, 24 bristles was the highest score observed. The high q_F at ADH, higher than in any of the other replicates, can perhaps be considered as further evidence of possible contamination. However, the low gene frequency at Est-6 indicates that the contamination, if it did occur, was not substantial. Apart from this one replicate there is little other evidence that contamination played any role in these results. The overall variance within replicates is smaller than that in the second experiment (Table 2.6) where, due to the use of visible markers, the possibility of contamination can be ruled out.

(ii) Experiment 2

(a) ADH

Table 2.4 and Figure 2.2a present the gene frequency at ADH. Two of the D and two of the C replicates have increased in q_F from the initial q_F of 0.58. Overall the frequency of the fast allele is higher in the D replicates by 0.05, but this is not significant. The mean square for differences between competitors is again very small. The variance between replicates is greater than that observed in the previous experiment. The variance due to drift in this experiment would be expected to be greater than that in the first experiment where 40 mated Kaduna females produced each new generation. In this experiment each generation was set up with 40 virgin females and 40 males and it is not possible to estimate exactly how many of these 40 females mated with competitor males and thus did not contribute to the offspring of the next Kaduna generation. Therefore the effective population size here would be smaller than in Experiment 1 and consequently a greater variance due to drift would be expected.

(b) Est-6

The results for this locus are presented in Table 2.5 and Figure 2.2b. An overall increase in $\bar{q}_F$ is apparent in both sets of replicates. However, as the variance between replicates is large these increases are not significant. Three of the four D replicates have increased in q_F, as expected with frequency dependent selection, but the fourth replicate, with a q_F of 0.10, is lower than any of the C replicates. Two of the C replicates have increased in frequency and two have decreased. The F_8^1 value

Table 2.4

(a) Observed and expected genotype numbers, chi square and gene frequency at ADH in Kaduna after 5 generations competition, during mating and the egg to adult stage, with DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.58 ± 0.03.

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	29	26	11	16	5	2	-	0.77±0.04
2	12	10	20	23	15	13	1.10	0.47±0.05
3	7	11	31	23	7	11	5.69**	0.50±0.05
4	30	29	14	17	4	3	-	0.77±0.04
								$\bar{q}_F$ = 0.63±0.08

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	24	26	23	18	1	3	-	0.74±0.04
2	10	8	19	23	19	17	1.43	0.41±0.05
3	26	24	15	18	5	3	-	0.73±0.05
4	8	9	25	23	13	14	0.36	0.45±0.05
								$\bar{q}_F$ = 0.58±0.09

** $p < 0.025$

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.0041	< 1.0	
Between replicates Within competitors	6	0.0293	11.27	< 0.001
Within replicates		0.0026		

Table 2.5

(a) Observed and expected genotype numbers, chi square and gene frequency at Est-6 in Kaduna after five generations competition, during mating and the egg adult stage, with DF (D Replicates) and C_3A (C Replicates).

Initial gene frequency = 0.32 ± 0.03

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	12	11	20	22	13	12	0.61	0.49±0.05
2	9	10	26	23	12	13	0.57	0.47±0.05
3	15	13	18	23	13	11	1.76	0.52±0.05
4	0	1	10	9	38	39	-	0.10±0.03
								$\bar{q}_F$ =0.40±0.10

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	10	10	21	21	12	12	0.00	0.48±0.05
2	2	1	11	13	35	34	-	0.16±0.04
3	22	21	17	21	7	5	1.76	0.66±0.05
4	3	3	16	17	29	29	-	0.23±0.04
								$\bar{q}_F$ =0.38±0.11

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.00031	<1.0	
Between replicates Within competitors	6	0.0461	17.73	<0.001
Within replicates		0.0026		

Table 2.6

(a) Means and standard errors of sternopleural bristle score in Kaduna after five generations competition, during mating and the egg to adult stage, with DF (D Replicates) and C_3A (C Replicates).

(b) Analysis of Variance.

(a)	D Replicates	C Replicates
Replicate No.		
1	17.08±0.32	17.70±0.30
2	18.68±0.30	16.98±0.37
3	17.33±0.31	17.73±0.42
4	17.08±0.26	17.65±0.29
Overall Mean	17.54±0.38	17.51±0.18

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.05	<1.0	
Between replicates Within competitors	6	14.37	3.38	<0.005
Within replicates	312	4.25		

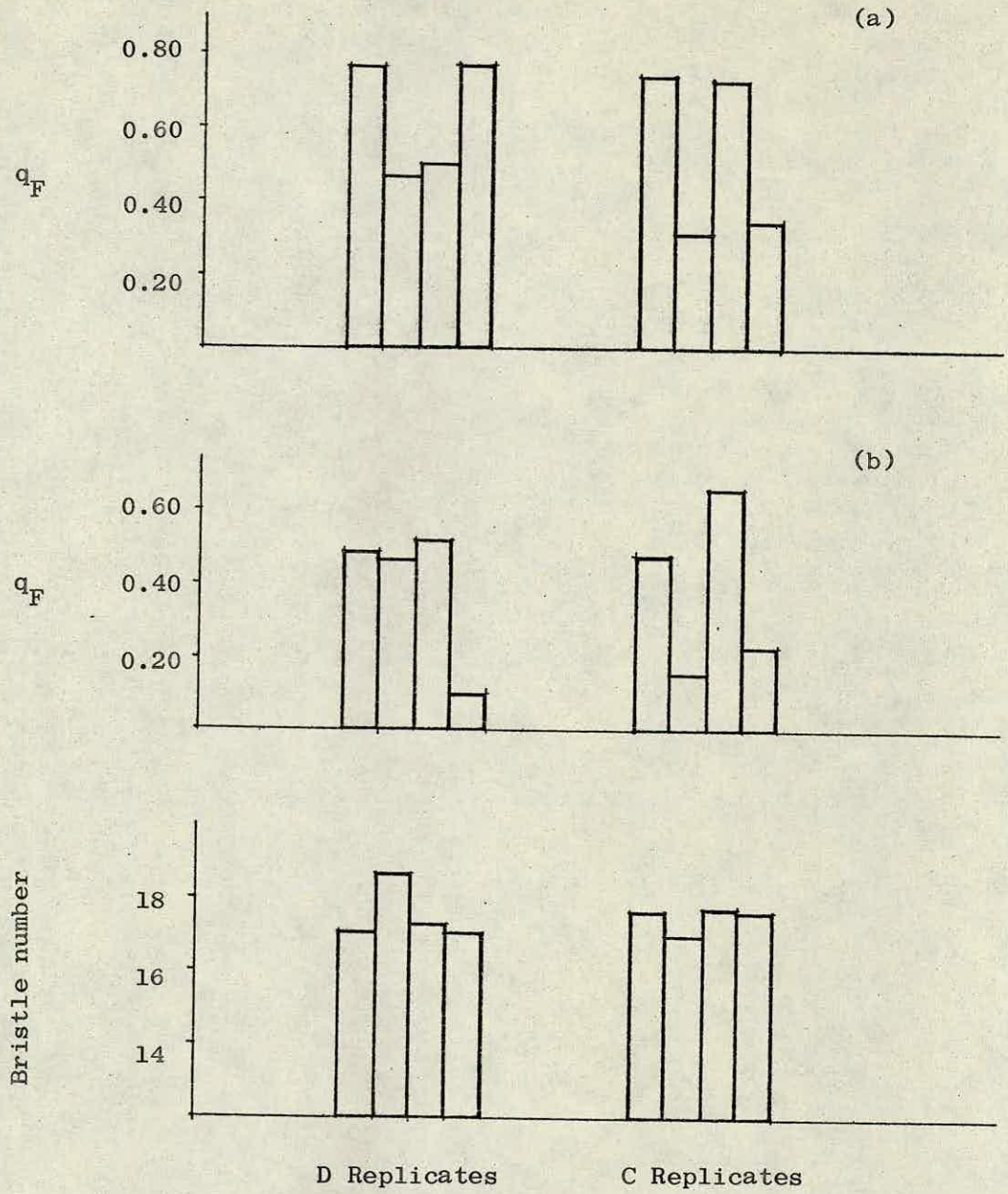

Fig. 2.2. Experiment 2. Gene frequency at ADH(a) and Est-6(b) and sternopleural bristle number (c).

in the analysis of variance for differences between competitors is less than 1.0 and for differences between replicates $F^{6}_{312} = 17.73$ ($p < 0.001$).

(c) Sternopleural bristles

These results are presented in Table 2.6 and Figure 2.2c. Sample sizes of 40 were again used. All the C replicates show a decrease in bristle score from that of the base population. However this does not appear to be the result of any frequency dependent selection as the same phenomenon is apparent in three of the D replicates. Overall there is no difference between competitors, the mean of the D replicates is 17.54 and that of the C replicates is 17.51.

(iii) Experiment 3

If frequency dependent selection is operating in this experiment through differential utilization of media, as proposed by Huang *et al.* then gene frequencies in the segregating population will be driven in the opposite direction from those in the monomorphic line, used to condition the media. So again q_F at the enzyme loci and bristle score would be expected to increase in the D replicates and decrease in the C replicates.

(c) ADH

Table 2.7.1 and 2.7.2 present the gene frequencies at ADH at density levels 1 and 2 respectively. Figure 2.3a presents histograms of the same results. A 2 x 2 cross classification analysis of variance, with two main effects, density and conditioning line, and an interaction, is presented with Table 2.7.2. From the table it can be seen that there is a significant density effect ($F^{1}_{20} = 13.26$; $p < 0.005$). The experiments for each density level were performed at

Table 2.7.1

Observed and expected genotype numbers, chi square and gene frequency at ADH in Kaduna emerging from media preconditioned, at density level 1, by DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.58±0.03.

D Replicates

Replicate No.	FF		FS		SS		χ^2_1	q_F
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.		
1	13	14	25	24	10	11	0.20	0.53±0.05
2	11	12	26	24	11	12	0.33	0.50±0.05
3	12	13	26	24	10	11	0.33	0.52±0.05
4	17	17	22	22	7	7	0.00	0.61±0.05
5	20	19	21	22	7	6	0.26	0.64±0.05
6	19	16	17	23	12	9	3.13	0.57±0.05
								$\bar{q}_F$ = 0.56±0.02

C Replicates

Replicate No.	FF		FS		SS		χ^2_1	q_F
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.		
1	21	21	22	21	5	5	0.05	0.67±0.05
2	23	23	20	21	5	5	0.05	0.69±0.05
3	16	16	24	23	8	8	0.04	0.58±0.05
4	18	16	20	23	10	8	1.14	0.58±0.05
5	18	19	25	22	5	6	0.63	0.64±0.05
6	19	20	24	22	5	6	0.40	0.65±0.05
								$\bar{q}_F$ = 0.64±0.02

Table 2.7.2

(a) Observed and expected genotype numbers, chi square and gene frequency at ADH in Kaduna emerging from media preconditioned at density level 2, by DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.64 ± 0.03.

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	29	27	14	18	5	3	-	0.75±0.04
2	33	31	11	15	4	2	-	0.80±0.04
3	19	18	22	23	7	7	0.10	0.63±0.05
4	18	17	22	23	8	8	0.10	0.60±0.05
5	24	25	21	19	3	4	-	0.72±0.05
6	24	25	21	19	3	4	-	0.72±0.05
							$\bar{q}_F$ =	0.70±0.03

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	23	20	15	21	8	5	3.96*	0.66±0.05
2	18	17	23	23	7	8	0.18	0.61±0.05
3	32	30	11	16	5	2	-	0.78±0.04
4	25	26	18	19	4	4	-	0.72±0.05
5	17	18	25	23	6	7	0.37	0.61±0.05
6	23	22	19	21	6	5	0.46	0.68±0.05
							$\bar{q}_F$ =	0.68±0.03

*$p < 0.05$

(b) Analysis of Variance

Source	df	M.S.	F	P
Density	1	0.0504	13.26	< 0.005
Conditioning Line	1	0.0033	<1.0	
Density x Conditioning Line	1	0.0150	3.97	> 0.05
Error	20	0.0038		

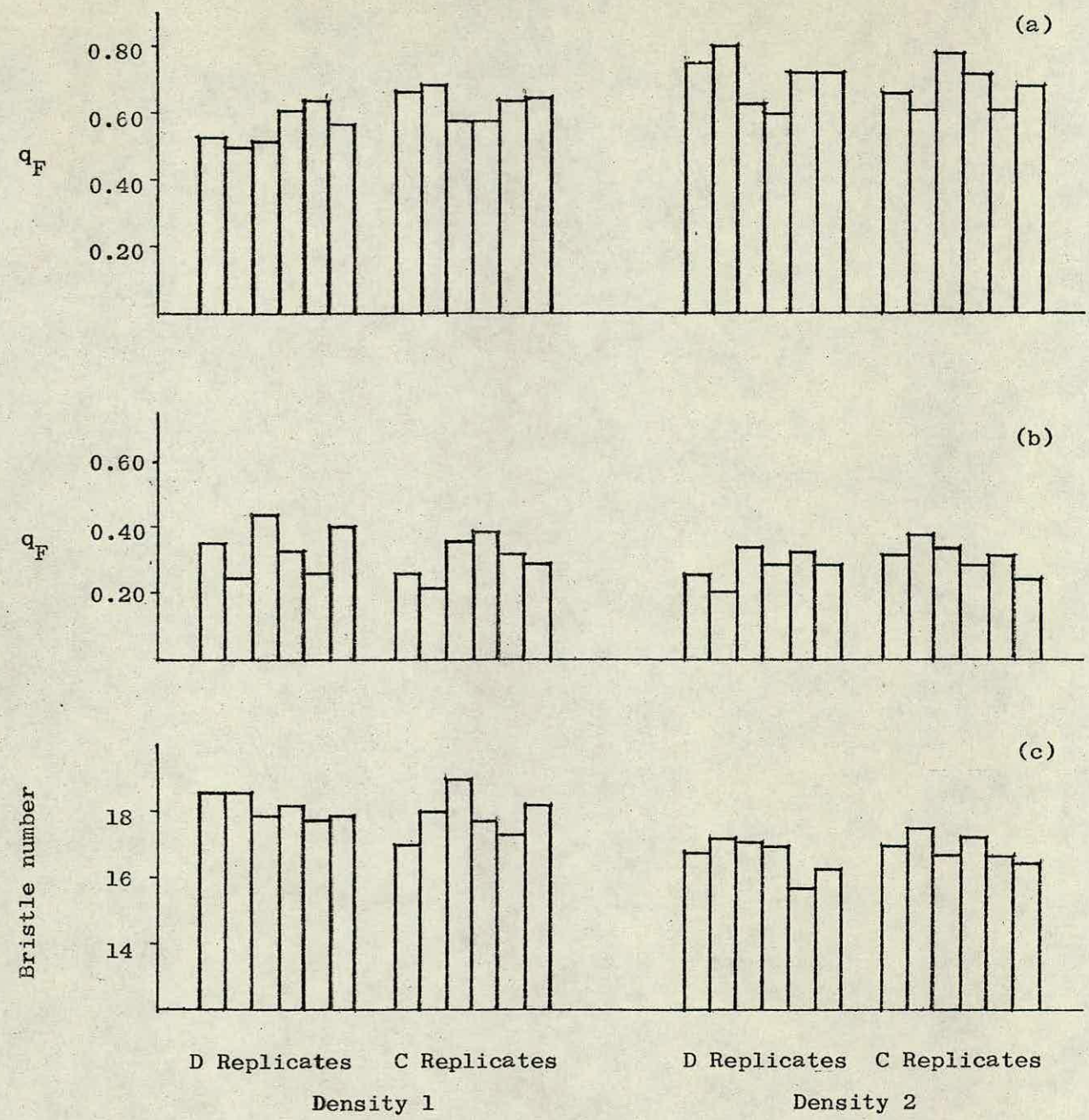

Fig. 2.3. Experiment 3. Gene frequency at ADH(a) and Est-6(b) and sternopleural bristle number (c).

different times. In the Kaduna cage population the gene frequency at ADH shows random fluctuations and when samples were taken for the lower density level the gene frequency was estimated as $q_F = 0.58 \pm 0.03$, whereas for density level 2 the estimate was $q_F = 0.64 \pm 0.03$. This difference in initial gene frequency may in part explain the significant density effect. However it may also be because the fast allele is favoured under the more severe competitive conditions of density level 2, where vials were set up with 300 larvae.

There is no evidence for frequency dependent selection, the F value for conditioning line is less than 1.0. The effect of any selection might be expected to be clearer when conditions are more severe. Looking at the results for density level 2 alone it can be seen that overall the D replicates are higher than the C replicates but only by 0.02 and the standard error of the difference is 0.04. There is some indication of an interaction between density and conditioning line but this is not significant. It is a result of the overall mean of the C replicates being higher than that of the D replicates at density level 1 while the reverse is true at density level 2.

(b) Est-6

Table 2.8.1 and 2.8.2 and Figure 2.3b summarize the results for this locus. From the analysis of variance (Table 2.8.2) it can be seen that neither the main effects nor the interaction approaches significance, and the mean square for conditioning line is again very small. The overall difference between the D and C replicates is in the direction expected with frequency dependent selection at density level 1 but not at density level 2.

Table 2.8.1

Observed and expected genotype numbers, chi square and gene frequency at Est-6 in Kaduna emerging from media pre-conditioned, at density level 1, by DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.31 ± 0.03.

D Replicates

Replicate No.	FF		FS		SS		χ^2_1	q_F
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.		
1	4	6	24	21	18	20	1.30	0.35±0.05
2	8	3	8	18	32	27	-	0.25±0.04
3	8	9	25	23	14	15	0.35	0.44±0.05
4	4	5	24	21	20	21	0.68	0.33±0.05
5	4	3	16	18	27	26	-	0.26±0.04
6	6	8	26	23	16	18	1.11	0.40±0.05
								$\bar{q}_F$ = 0.34±0.03

C Replicates

Replicate No.	FF		FS		SS		χ^2_1	q_F
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.		
1	5	3	15	17	28	26	-	0.26±0.04
2	2	2	17	16	29	29	-	0.22±0.04
3	11	6	13	22	24	19	9.16***	0.36±0.05
4	6	7	25	22	17	18	0.61	0.39±0.05
5	4	5	23	21	21	22	0.44	0.32±0.05
6	5	4	18	19	25	24	-	0.29±0.05
								$\bar{q}_F$ = 0.31±0.03

*** $p < 0.01$

Table 2.8.2

(a) Observed and expected genotype numbers, chi square and gene frequency at Est-6 in Kaduna emerging from media pre-conditioned, at density level 2, by DF (D Replicates) and C_3A (C Replicates). Initial gene frequency = 0.30 ±0.03.

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	4	3	16	18	26	25	-	0.26±0.04
2	1	2	18	16	28	29	-	0.21±0.04
3	5	6	23	22	20	21	0.26	0.34±0.05
4	5	4	18	20	25	24	-	0.29±0.05
5	9	5	14	21	25	21	6.29**	0.33±0.05
6	3	4	21	19	24	25	-	0.28±0.05
								$\bar{q}_F$ = 0.29±0.02

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	3	5	23	19	19	21	1.83	0.32±0.05
2	5	7	26	23	17	19	1.17	0.38±0.05
3	8	6	17	22	23	21	1.99	0.34±0.05
4	3	4	22	20	23	24	-	0.29±0.05
5	3	5	25	21	20	22	1.74	0.32±0.05
6	3	3	17	17	26	26	-	0.25±0.04
								$\bar{q}_F$ =0.32±0.02

** $p < 0.025$

(b) Analysis of Variance

Source	df	M.S.	F	P
Density	1	0.0051	1.37	>0.25
Conditioning Line	1	0.00035	<1.0	
Density x Conditioning Line	1	0.0030	<1.0	
Error	20	0.0037		

(c) Sternopleural Bristles.

These results are presented in Table 2.9 and Figure 2.3c. The mean of each replicate is estimated from a sample of 30 flies, 15 males and 15 females. The analysis of variance is performed on means, giving a total of 24 observations, 6 for each density x conditioning line combination. The effect of density is highly significant ($F^{1}_{20} = 36.14$; $p < 0.001$). This density effect is a result of over-crowding, which causes a reduction in bristle score and this has clearly occurred at the higher density, in both D and C replicates alike. The other two sources of variation, conditioning line and interaction, are both non significant.

Table 2.9

(a) Means and standard error of sternopleural bristle score in Kaduna emerging from media preconditioned, at density levels 1 and 2, by DF (D Replicates) and C_3A (C Replicates).

(b) Analysis of Variance.

	Density 1		Density 2	
Replicate No.	D Replicates	C Replicates	D Replicates	C Replicates
1	18.57±0.46	17.00±0.28	16.77±0.33	16.97±0.32
2	18.60±0.31	18.03±0.31	17.20±0.31	17.53±0.30
3	17.93±0.28	19.00±0.29	17.07±0.35	16.73±0.32
4	18.27±0.29	17.83±0.33	16.97±0.25	16.83±0.27
5	17.77±0.24	17.33±0.34	15.73±0.24	16.67±0.27
6	17.90±0.28	18.23±0.36	16.30±0.30	16.50±0.24
Overall mean	18.17±0.15	17.90±0.29	16.67±0.23	16.87±0.15

Analysis of Variance

Source	df	M.S.	F	P
Density	1	9.614	36.14	<0.001
Conditioning Line	1	0.007	<1.0	
Density x Conditioning Line	1	0.329	1.24	>0.25
Error	20	0.266		

IV DISCUSSION AND CONCLUSIONS

The detection of selection is generally considered as positive evidence for selection but failure to find selection does not necessarily disprove its existence in any particular case. The results presented here then can be considered in either of two ways. They may be thought of as simply a failure to find frequency dependent selection or as evidence that frequency dependent selection, of the type proposed by Kojima, is not operating at the loci studied in the Kaduna population. In order to conclude the latter, a closer examination of the experimental design and the results obtained, in comparison to those of Kojima, is necessary.

Kojima and Yarbrough (1967), in their studies of the Est-6 locus, set up cultures, using mated females at four different frequencies of the fast allele. These frequencies were 0.70, 0.50, 0.30 and 0.15; 0.30 being the equilibrium gene frequency in the base population. If frequency dependent selection is operating at the locus, and the gene frequency is disturbed from its equilibrium, the allele present at a frequency above its equilibrium value will be selected against while the other allele will be favoured. So the situation where, for example, the fast allele is above its equilibrium can be represented simply as follows

Genotype	FF	FS	SS
Frequency	q^2	2pq	p^2
Selective value	1-s	1	1+s

and the change in gene frequency in one generation is given by

$$\Delta q = -spq.$$

For frequencies of the fast allele below equilibrium, the selective values of the two homozygotes will be reversed and the change in gene frequency will be positive. The paper presents tables of the expected and observed proportions of the three genotypes emerging from the different cultures. The expected and observed q_F for the two extreme frequencies, calculated from Kojima and Yarbrough's tables, along with the observed change in gene frequency, Δq, and the selective coefficient, s, necessary to cause this change are presented below. The expected q_F is adjusted for the differential egg laying capabilities of the three genotypes.

q_F(exp.)	q_F(obs.)	Δq	s
0.710	0.610	-0.100	0.49
0.170	0.300	+0.130	0.92

It cannot be assumed immediately that the observed changes in gene frequency were solely the result of frequency dependent selection. Heterozygote superiority would also cause changes in gene frequency of this nature. However the authors have excluded heterosis as an important factor. The viability estimates obtained for the three genotypes did not fit a model of constant selective values but were instead frequency dependent. They concluded that frequency dependent selection was responsible for the changes in gene frequency.

Kojima and Tobari (1969b) performed similar experiments with ADH. Here the equilibrium in the base population was $q_F = 0.60$ and three gene frequencies were studied. The details of the two

extreme gene frequencies are given below.

q_F(exp.)	q_F(obs.)	Δq	s
0.750	0.716	-0.034	0.18
0.250	0.276	+0.026	0.14

Again the possibility of heterozygote superiority playing a part was discounted after failure to fit a model with constant selective values.

Turning now to the experiments reported here involving these two loci, two questions pose themselves. First, what were the extreme gene frequencies generated through competition with the two competitor lines, and secondly what order of selective coefficients could have been detected?

In the two experiments run over five generations each generation was set up with a ratio of 1:4 of Kaduna to competitor. The gene frequency in the culture as a whole, q_F, can be calculated as

$$q_F = 0.2\ (q_K) + 0.8\ (q_{comp.})$$

where q_k is the frequency of the fast allele in Kaduna and q_{comp} the frequency of fast in the competitor. The equilibrium gene frequencies in the Kaduna populations are the same as those in Kojima's base populations; 0.60 for ADH and 0.30 for Est-6. Thus with DF as competitor, the frequency of the fast allele at ADH is

$$q_F = 0.2\ (0.60) + 0.8\ (0.0) = 0.12$$

and at Est-6 is

$$q_F = 0.2\ (0.30) + 0.8\ (0.0) = 0.06.$$

With C_3A as the competitor the frequency at ADH is

$$q_F = 0.2\ (0.60) + 0.8\ (1.0) = 0.92$$

and at Est-6 is

$$q_F = 0.2\ (0.30) + 0.8\ (1.0) = 0.86.$$

Thus the extreme frequencies here, for both loci, were further removed from their equilibrium values than those used by Kojima. Therefore, if the selective value of an allele increases with decreasing frequency and vice versa, the selective coefficients operating here might be expected to be stronger than those observed by Kojima. Which leads to the second question: What are the minimum selective coefficients that could have been detected by these experiments? For significant differences, between competitors, in gene frequency, to be detected the mean difference must be greater than twice the standard error of the difference.

$$\frac{\text{Difference}}{\text{S.E. difference}} = \frac{\Delta q}{\sqrt{\sigma^2_{(\bar{q}_D - \bar{q}_C)}}} > 2.$$

where $\bar{q}_D$ and $\bar{q}_C$ are the mean gene frequencies of the D and C replicates respectively, and the variance of the difference

$$\sigma^2_{(\bar{q}_D - \bar{q}_C)} = \frac{2\sigma^2_q}{R}$$

since the variance of gene frequency, σ^2_q is expected to be the same

in both sets of R replicates. The variance of gene frequency among replicates after t generations, expected due to random drift alone, given by Falconer (1960), is

$$\sigma_q^2 = p_o q_o \left[1 - \left(1 - \frac{1}{2N}\right)^t\right]$$

where p_o and q_o are the initial frequencies of the two alleles and N the effective population size. The appropriate value for t here is 6. This is because there were five generations each of which was set up using 80 individuals, followed by a final sampling of 48 individuals for electrophoresis. However an estimate of the effective population size, for experiment 1, of 40 was considered more reasonable, and for experiment 2, of 30. The effective population size in experiment 2 would be expected to be smaller because of the use of virgin females, as previously mentioned.

The overall difference in gene frequency, between D and C replicates, at the end of the t generations, Δq_t, is given by

$$\Delta q_t = \Delta q_{Dt} + \Delta q_{Ct}$$

where Δq_{Dt} and Δq_{Ct} are the changes in gene frequency in the D and C replicates. The change in gene frequency in one generation (using the same model as before, with 1+s, 1, and 1-s the selective values for FF, FS and SS, when F is the favoured allele and vice versa when S is the favoured allele) is given by

$$\Delta q = spq.$$

Assuming that spq will be the same in each generation, then the change after t generations can be simplified to tspq. If Δq_{Dt} and Δq_{Ct} are equal

in magnitude though opposite in direction, the overall difference

$$\Delta q_t = 2tspq.$$

As frequency dependent selection is under consideration, the assumption of a constant s should perhaps be justified. In this case s is dependent on q_F, the frequency in the culture as a whole, which, as pointed out previously, is given by

$$q_F = 0.2\ (q_K) + 0.8\ (q_{comp}).$$

Each generation is set up with these proportions and only q_K will alter from generation to generation. Any change in q_K will bring about a change of only one-fifth the magnitude in q_F, so the assumption of a constant s over the five generations can be expected to hold reasonably well. Thus the values of s which could be detected by the two five generation experiments can be estimated from

$$\frac{2tspq}{\sqrt{\frac{2\sigma_q^2}{R}}} > 2$$

where s is the only unknown, and for the conditioned media experiment, where t = 1, from

$$\frac{2spq}{\sqrt{\frac{2\sigma_q^2}{R}}} > 2$$

where $\sigma_q^2 = \frac{p_o q_o}{2N}$ and 2N = 96, the number of genes sampled. It is not possible to make such estimates for sternopleural bristles. To arrive at an estimate of a single s for a quantitative character would

require such unrealistic assumptions as would render the estimate meaningless. Table 2.10 gives the values of s, estimated for the two enzyme loci from the formulae given above.

Table 2.10

Selective coefficients detectable in the three experiments

Experiment No.	Locus	N	R	t	$p_o q_o$	$E(\sigma_q^2)$	S
1	ADH	40	5	5	0.24	0.0174	0.069
	Est-6	40	5	5	0.21	0.0153	0.078
2	ADH	30	4	5	0.24	0.0230	0.089
	Est-6	30	4	5	0.21	0.0202	0.095
3	ADH	48	6	1	0.24	0.0025	0.121
	Est-6	48	6	1	0.21	0.0022	0.128

From the table it can be seen that, in all three experiments, the selective coefficients detectable, are lower than those found by Kojima, given before. This is particularly true in the case of Est-6, where Kojima found selective coefficients of 0.49 and 0.92 for the two extreme frequencies studied. The conditioned media experiment can be seen to be the least powerful of the three experiments in detecting selection, but even here the selective coefficients are lower than those of Kojima. However, the results presented here show no evidence whatsoever of any frequency dependent selection and it may reasonably be concluded that, frequency dependent selection, of the type found by Kojima, is not in fact operating at these loci in the Kaduna population.

If frequency dependent selection is not playing a part in these experiments, can it then be concluded that the observed variance in gene frequency, and bristle score, is a result of drift alone? If heterozygote superiority is responsible for the maintenance of variability, at the loci studied, no change from the initial values would have been expected. Can the effects of heterotic selection, striving to maintain the equilibrium values, be distinguished from the forces of random drift tending to cause dispersion?

Cavalli-Sforza (1966) proposed a method whereby the effects of selection and drift might be distinguished. If drift alone is responsible for the observed variance of gene frequencies, among replicates after t generations then, as given previously,

$$\sigma_q^2 = p_o q_o \left[1 - \left(1 - \frac{1}{2N}\right)^t\right]$$

and this formula, expressed in terms of the inbreeding coefficient, F, becomes

$$\sigma_q^2 = p_o q_o F.$$

Cavalli-Sforza (1966) has proposed that, $F = \frac{\sigma_q^2}{p_o q_o}$, referred to as the standardized variance of gene frequency or the Wahlund variance, can be used to distinguish between drift and selection. Drift will affect all loci in a similar manner, while selection would be expected to act differently on different loci. If drift alone is responsible for the variance among replicates observed in the two five generation experiments, then estimates of F obtained from the three loci will be homogeneous. Selection, on the other hand, at one or all of the loci, will result in heterogeneity among the F estimates. Selection can

result in a larger or smaller estimate of F, than that expected with drift alone, depending on the type of selection. Generally speaking, if selection, at a locus, is acting differently in different replicates, then an inflated F value for that locus will result. If selection is acting similarly in all replicates then the value of F obtained for the locus will be less than expected. Homogeneity of estimates of effective population size can be used in the same way to test for selection (Krimbas and Tsakas, 1970). F and N can be estimated for sternopleural bristles as well as the two enzyme loci. The expected variance among replicates, σ_b^2 in the analysis of variance, for a quantitative character (with little non-additive genetic variance, as bristles) subjected to slow inbreeding, is given by Falconer (1960), as

$$\sigma_b^2 = 2F\sigma_a^2$$

where σ_a^2 is the genetic variance in the base population. Thus for a quantitative character $F = \frac{\sigma_b^2}{2\sigma_a^2}$. In the Kaduna population the heritability of sternopleural bristle score, $h^2 = 0.49$ and the phenotype variance, $\sigma_p^2 = 3.71$, so $\sigma_a^2 = h^2\sigma_p^2 = 1.81$. Table 2.11 gives the product of the initial frequencies $p_o q_o$ (or $2\sigma_a^2$ in the case of bristles), the observed variance between replicates, σ_p^2, corrected for the variance within replicates, (or σ_b^2), and the values of F and N estimated from these, in the two five generation experiments.

Table 2.11

Standardized Variances and Effective Population Sizes

Experiment No.	Locus	$p_o q_o$	σ_q^2	F	N
1	ADH	0.2451	0.0123	0.050	49
	Est-6	0.2100	0.0187	0.089	27
	Bristles	3.62	0.3780	0.104	23
2	ADH	0.2436	0.0233	0.096	25
	Est-6	0.2176	0.0373	0.171	14
	Bristles	3.62	0.2500	0.069	35

Lewontin and Krakauer (1973) developed statistical tests for the homogeneity of such F and N estimates. As testing F or N estimates will yield the same conclusions, F estimates only will be dealt with here. The theoretical variance of F is given by Lewontin and Krakauer (1973) as

$$\sigma_F^2 = \frac{k\bar{F}^2}{n-1}$$

where n is the number of replicates, and k = 2 when the underlying distribution of p is binomial and is closer to 1 for a uniform or U shaped distribution. With only ten replicates in which to estimate p it is difficult to conclude anything about the distribution of p, but it is probably closest to a binomial as only five generations of sampling have occurred. A value of 2 was then used to calculate the theoretical variance of F, which is given below with the observed variance of F and the ratio of observed to expected, for the two experiments.

	Theoretical variance	Observed variance	Ratio:Obs./Exp.
Expt. 1	0.001458	0.000777	0.53
Expt. 2	0.003584	0.002793	0.78

Ratios of observed to expected of less than 1.0, in both cases, indicate no evidence of heterogeneity among the observed F values. It must however, be stressed that, with only three estimates of F for each experiment, it would be unwise to draw any conclusions about the presence or absence of selection. Also, as shown by Nicholas (1974), with observed F values of less than 0.1, which four of the above are, it is unlikely that the effect of selection could be detected. However, even though the data are insufficient to draw any definite conclusions, certain trends are perhaps apparent. In both experiments the F value obtained for Est-6 is greater than that obtained for ADH. This could perhaps be interpreted as an indication of some form of selection tending to maintain the equilibrium value at the ADH locus. Bristles yield the highest F value in the first experiment and the lowest in the second. The Experiment 1 value is perhaps more reliable. From the bristle scores for Experiment 2 given in Table 2.6, it can be seen that three of the C replicates have almost identical means and there seems to be little other than chance to which this can be attributed. The larger F values, and lower N values, for Experiment 2 were as expected from the experimental design. The valuesof N indicate that the effective population sizes, used in estimating the selective coefficients detectable, (Table 2.10), were reasonable.

This search for frequency dependent selection at specific loci, has yielded results in complete contradiction to those of Kojima and

Yarbrough (1967), for Est-6, and Kojima and Tobari (1969b), for ADH. Such selection is not perhaps then as widespread as these authors have proposed. Frequency dependence has not previously been investigated for sternopleural bristle number, and its absence in these results is in line with Robertson's (1970) belief that bristles are a trivial character not subject to strong natural selection. The results for the three loci are not such as to rule out other forms of selection, among which it is necessary to include frequency dependent selection with selective coefficients of the order of 0.01 or less. Such selective coefficients may indeed be the rule rather than the exception and if so, many experiments, including those reported here, could be said to be performed in a spirit of naive optimism.

CHAPTER 3

GENERAL FREQUENCY DEPENDENT SELECTION

CHAPTER 3

GENERAL FREQUENCY DEPENDENT SELECTION

I INTRODUCTION

Huang et al. (1971) and Kojima and Huang (1972) presented results which pointed to a mechanism through which frequency dependent selection was operating. They found that lines homozygous for the fast allele at Est-6 showed the highest larval survival in media conditioned by lines homozygous for the slow allele and vice versa. They proposed that the conditioning process in some way caused either depletion of essential nutrients or left behind deleterious by-products, thus reducing the chance of survival of the same genotype in that medium. Thus a particular genotype when rare (i.e. in heterotypically conditioned media) was at an advantage and this advantage disappeared when this genotype became common (i.e. in homotypically conditioned media). Kojima and Huang (1972) also stressed the importance of density in the establishment of frequency dependent selection, such selection could not be expected to operate when population density was low.

The aim of the first two experiments reported here was to use this procedure of conditioning media, together with high density levels, in a search for frequency dependent selection of a multigenic origin. It was concluded in the preceding chapter that frequency dependent selection was either not operating at the loci studied, or that the strength of selection involved was such as to render detection at a single locus almost impossible. However perhaps the cumulative effects of selection at many loci could be detected using highly selected or inbred lines. If frequency dependent selection, oper-

ating through differential utilization of media, is a widespread phenomenon then lines differing at many loci should show decreased viability in homotypically conditioned media relative to viability in heterotypically conditioned media.

The results obtained in the first two experiments prompted the third experiment, which in turn lead to the final experiment in this section. The four experiments will be reported in the sequential manner in which they were performed.

II EXPERIMENTAL DESIGN AND RESULTS

(i) Experiment 4

(a) Materials and Methods

The two highly selected Kaduna lines DF and C_3A, previously described in Ch.2(II), were each used to condition media and the viability of each line was measured in both types of media. The C_3A line was again one homozygous for claret eye, while the DF line used here was homozygous for the apricot allele at the white eye sex linked locus.

The method of conditioning media was as described for Experiment 3, Chapter 2, and was exactly parallel in quantity and type of medium, culture conditions and general procedure to that of Kojima and Huang (1972). The density of larvae used however was different. The highest densities used by these authors were 150 to condition followed by 150, and 100 to condition followed by 200. Here 150 were used to condition 5cc of cornmeal molasses medium in vials and viability was measured on 220. The survival of each line was measured in four replicates of homotypic media and four replicates of heterotypic media. The experiment then consisted of 16 vials in all, four for each mediumxline combination. Vials were maintained at 25°C and all emerging adults were counted.

(b) Results

Table 3.1 presents the number of adults of each line emerging from each type of conditioned medium, together with a 2 x 2 cross classification analysis of variance. As survival percentages covered a wide range an arcsin transformation was used and the analysis is presented in angles. Frequency dependent selection in these results

Table 3.1

Viability of DF and C_3A in homotypically and heterotypically conditioned media. (a) Mean number of adults emerging from 220 first instar larvae. (b) Analysis of variance in angles.

(a)

Media conditioned by:	Line DF	Line C_3A
DF	87.75±2.56	158.00±2.68
C_3A	79.25±7.19	154.50±2.02

(b)

Source	df	MS	F	P
Line	1	1533.11	283.75	< 0.001
Conditioned media	1	12.92	2.39	> 0.25
Line x media interaction	1	1.06	< 1.0	
Error	12	5.40		

would be manifest in a significant interaction, as the viability of each line would be expected to be lowest in homotypic media. There is no evidence for such an interaction, the mean square for this term is less than 1.0. There is a highly significant difference in viability between the two lines ($F^{1}_{12} = 283.75$; $p < 0.001$), but as conditioners there is no difference between the lines $F^{1}_{12} = 2.39$; $p > 0.25$).

As no evidence for frequency dependent selection was found using these two lines, it was decided to extend the study to cover more lines and at the same time to increase the density level.

(ii) Experiment 5

(a) Materials and Methods

Six lines in all were used and the information regarding their genotype at Est-6 and ADH is summarized in Table 3.2.

Table 3.2

Allele frequencies at ADH and Est-6

	ADH		Est-6	
Line	F	S	F	S
DF	0.00	1.00	0.00	1.00
C_3A	1.00	0.00	1.00	0.00
3	0.46	0.54	0.00	1.00
4	0.00	1.00	1.00	0.00
5	0.54	0.46	0.21	0.79
6	1.00	0.00	0.00	1.00

DF and C_3A are as described for the previous experiment. Lines 3 and 5 were originally derived from the standard Kaduna population and have been maintained with 20 pairs of parents for over 400 generations.

Line 4 was derived from Kaduna by over 400 generations of full sib mating, and line 6 is an inbred white eyed Oregon R line. The conditioning procedure was as before, with 150 larvae used to condition but followed this time by 300 larvae per vial. 10 vials, each containing 5cc cornmeal molasses medium, were conditioned by each line. Five of these were used to measure the viability of the same line again, and one vial was used for each of the other five lines. Thus the viability of each line was measured in five vials of homotypic media and in five vials of heterotypic media. Vials were maintained at 25°C and all emerging adults were counted.

(b) Results

Table 3.3a presents the number of adults, of each line, emerging from 300 larvae cultured in conditioned media. Each of the figures on the diagonal (where the medium is homotypically conditioned) is the mean of five replicates, the results of which are given individually in Table 3.3b. The analysis of variance in angles is presented in Table 3.4. The analysis was performed using least squares and the statistical model was as follows

$$X_{ijk} = \mu + c_i \delta_{ij} + m_i + l_j + e_{ijk}$$

where X_{ijk} is the k^{th} observation (with $k = 5$ when $i = j$ and $k = 1$ when $i \neq j$) of the j^{th} line in the i^{th} conditioned medium. $c_i \delta_{ij}$ is the effect of homotypic medium, with $\delta_{ij} = 1$ when $i = j$ and $\delta_{ij} = 0$ when $i \neq j$.

Frequency dependent selection of a multigenic origin would be manifest in two facets of the results. The first and most direct effect would be that of homotypic media. The second would be the

Table 3.3

Numbers of adults emerging from 300 first instar larvae in conditioned media. The numbers on the diagonal in (a) are the means of five replicates in homotypically conditioned media, given individually in (b).

(a)

Media Conditioned by:	Line DF	C_3A	3	4	5	6	Average percent survival for all lines
DF	114	214	238	207	261	214	69
C_3A	112	205	230	213	238	252	69
3	136	219	237	216	239	253	72
4	134	216	249	234	240	276	75
5	107	200	245	225	259	246	71
6	125	204	253	247	251	237	73
Average percent survival on all media	40	70	81	75	83	82	

(b)

Line DF	C_3A	3	4	5	6
128	198	238	236	253	209
124	207	241	231	262	254
99	204	230	223	253	247
110	197	229	228	254	236
109	218	247	250	275	241

Table 3.4

Analysis of variance in angles

Source	df	M.S.	F	P
Lines as survivors	5	1038.07	124.77	<0.001
Lines as conditioners	5	17.22	2.07	>0.05
Homotypic media (overall)	1	0.28	<1.0	
Homotypic media (between lines)	5	15.58	1.87	>0.10
Interaction between survivors and conditioners	19	9.80	1.37	>0.10
Between replicates (of homotypic media)	24	7.15		
Pooled error	43	8.32		

existence of any interaction between a line as a survivor and another line as a conditioner. As there is no indication of any such interaction, this variation is combined with that between homotypic replicates to give an overall error with 43 degrees of freedom for the rest of the table. The test for the effect of homotypic media is split into two in the analysis, testing first for differences in the effect of homotypic medium between lines, with 5 degrees of freedom. The result of this test is non significant as is the result of the test for an overall difference in viability in homotypic and heterotypic media. There is a highly significant difference in the viability of the six lines, mainly due to the extremely low value of line 1. As conditioners the difference between the lines is not significant though it is interesting to note that the two lines with the lowest viabilities, DF and C_3A, had the most detrimental effects as conditioners. However as there is no difference between viability in homotypic media and viability in heterotypic media, a general frequency dependent effect does not appear to be involved.

The use of conditioned media then had failed to demonstrate frequency dependent selection of a multigenic origin. Perhaps it was necessary for larvae to be present in a culture at the same time, competing for the available nutrients, before such selection would be evident. The aim of the third experiment was to test this hypothesis.

(iii) Experiment 6

(a) Materials and Methods

Four of the six lines were used. The two highly selected lines w^aDF and caC_3A were competed (Series A); and line 6, the white eyed

Oregon R inbred line, was competed against Kaduna line 3 (Series B). In both series emerging adults could be distinguished on the basis of eye colour. The design was the same for both series and consisted of measuring the viability of each line at five different frequencies, 9:1, 7:3, 1:1, 3:7 and 1:9. Three replicates were set up for each of the extreme frequencies, and two replicates for the three intermediate frequencies. The experiment was again performed in vials containing 5cc of cornmeal molasses medium and to ensure intense competition a total of 500 larvae per vial was used. First instar larvae, one to four hours old, were transferred to vials, taking first a certain percentage of the total number of larvae of one line to be used followed by the same percentage of the competing line and repeating the process until 500 larvae in all had been transferred. Vials were maintained at 25^{o}C and all emerging adults were counted.

(b) Results

Table 3.5 presents, for both series, the mean percentage survival of each line at the five frequencies tested, together with the regression coefficients obtained when the survival of each line was regressed against the frequency of one of them. The regressions for Series A and Series B are presented in Figure 3.1a and 3.1b respectively.

It is difficult to statistically test for differences in the survival of a particular line at different frequencies. This is because the variation between replicates at each frequency is different, being very much greater when only 50 larvae of the line are present than when 450 are present. If survival overall is frequency dependent, then the regression of a line on its own frequency (as presented in Figure 3.1) will be negative while the regression of the other line will be positive. For Series B this has occurred, however neither

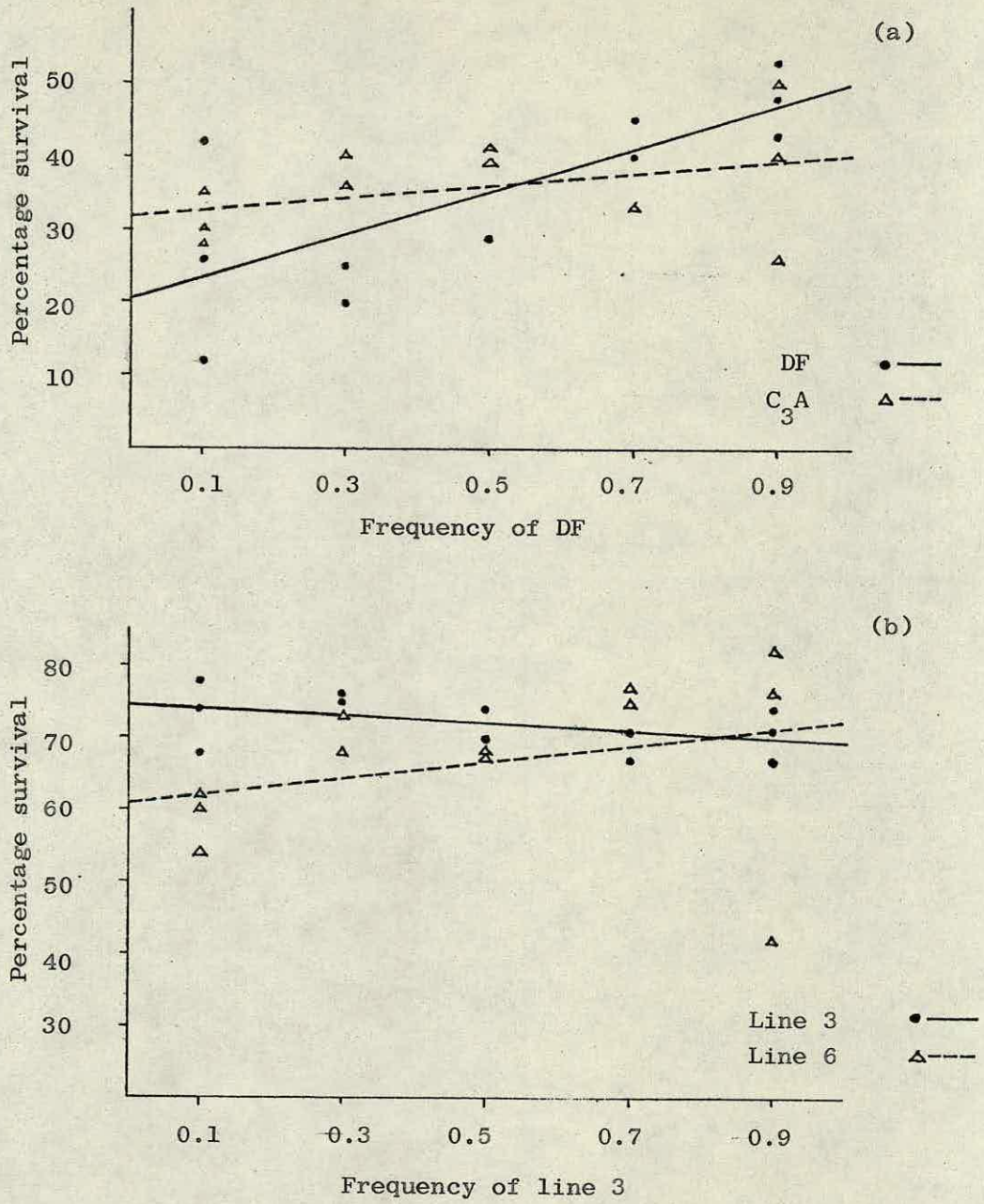

Fig. 3.1. Experiment 6. Regression of percentage survival on frequency in Series A(a) and Series B(b).

regression is significant. In Series A both regression coefficients are positive and that obtained for DF is significant. However this significant frequency dependence is not the type that will maintain polymorphism, as the viability of DF increases as its frequency rises. Although the results do not demonstrate a significant overall frequency dependence, with advantage of the rare genotype, it can be seen from Table 3.5 that both C_3A and line 6 have lowest viability at highest frequency. It would appear then that these results indicate some tendency towards frequency dependence, though any conclusions about the importance of the phenomenon must take into account another and very striking form of interaction between genotypes which occurred in this experiment.

Closer examination of the emergence pattern in both series reveals significant differences in developmental time which are not present when these lines develop in pure cultures. These results are summarized in Table 3.6 and Figure 3.2. In all cases there is a significant difference in developmental time of the two competing lines. Line 3 emerges about five days earlier than line 6 at all frequencies. In Series A DF emerges first though the time difference is not as great as that observed in Series B. Also developmental time in both lines tends to increase as the frequency of the first emerging line increases. This tendency is again more striking in Series B.

Percentage survival over all frequencies is compared, in Table 3.7, to that observed for these lines in Experiment 5 (Table 3.3a). The higher density used in this experiment reduces viability in the later emerging lines more than in the earlier emerging lines. In Series A the viability of C_3A is only 35% compared with 70% in the

Table 3.5

Mean percentage survival at different frequencies

Frequency of DF or line 3	Series A			Series B		
	DF	C_3A	Combined	3	6	Combined
0.1	27.3	31.1	30.7	73.3	58.7	60.2
0.3	22.7	38.0	33.4	75.7	70.6	72.1
0.5	34.0	40.2	37.1	72.0	68.4	70.2
0.7	42.1	35.3	40.1	69.1	75.7	71.1
0.9	47.9	38.7	46.9	70.6	66.7	70.2
Overall frequencies	40.6	35.0	37.9	71.1	65.2	68.2
Regression Coefficient	+0.29	+0.07		-0.05	+0.11	
Standard error	±0.08	±0.06		±0.03	±0.11	

Table 3.6

Mean developmental time, in days, at different frequencies

Frequency of DF or line 3	Series A		Series B	
	DF	C_3A	3	6
0.1	11.80±0.23	13.54±0.10	10.64±0.14	15.64±0.13
0.3	11.85±0.17	14.08±0.13	11.17±0.09	16.40±0.11
0.5	11.68±0.13	14.30±0.12	11.90±0.12	16.30±0.14
0.7	13.18±0.13	15.51±0.17	13.18±0.13	18.94±0.07
0.9	13.01±0.08	13.90±0.22	14.12±0.14	19.28±0.29

Table 3.7

Overall percentage survival

Line	Experiment 5	Experiment 6
DF	40	41
C_3A	70	35
3	81	71
6	82	65

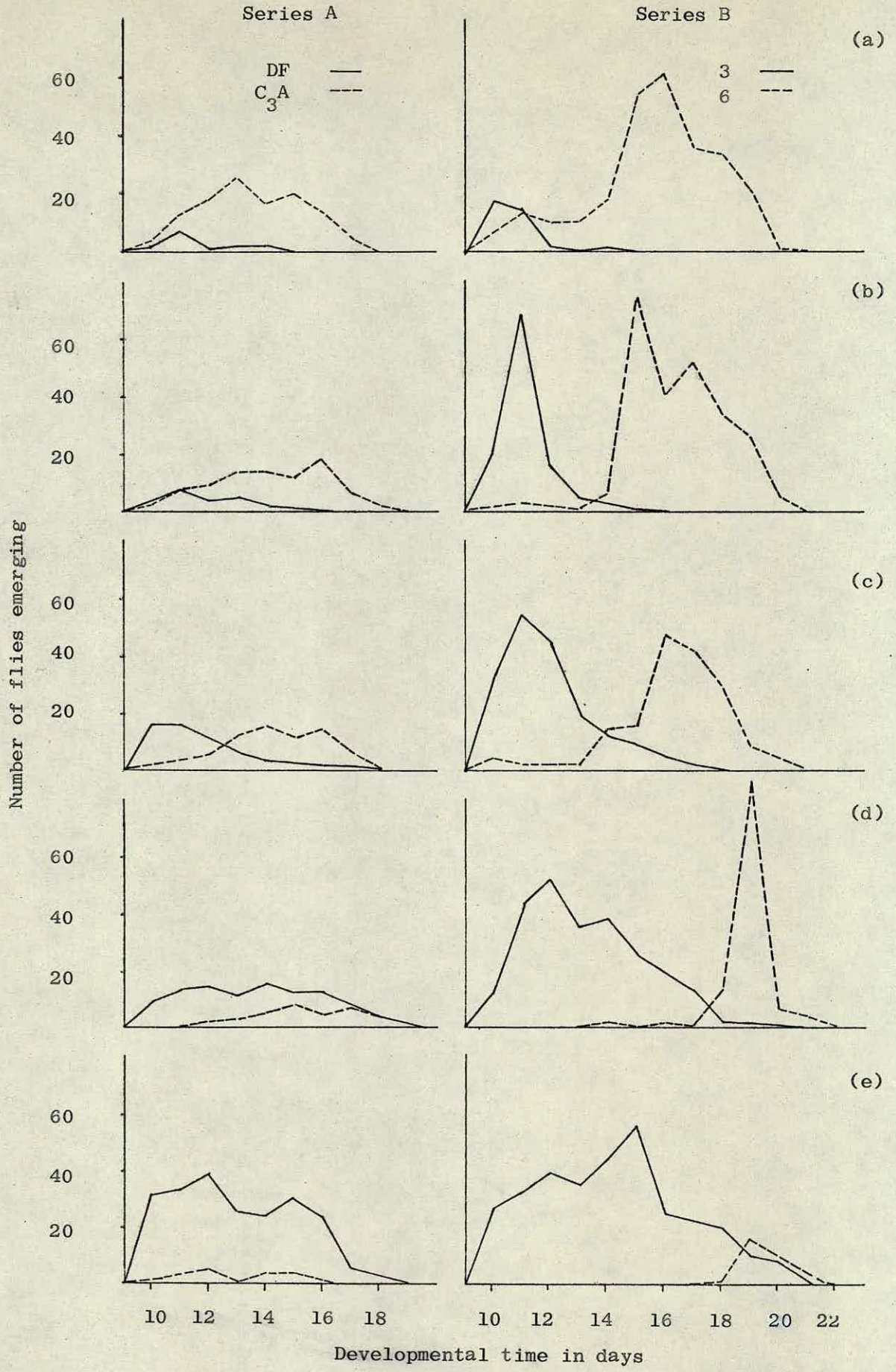

Fig. 3.2. Experiment 6. Developmental time at different frequencies. The frequency of DF and line 3 is increasing from (a) to (e).

previous experiment, while viability in the competing line, DF does not appear to have been affected.

C_3A and line 6 are the two lines in which it was noted that viability is lowest at highest frequency. Thus the results indicate that the slower emerging lines show the greatest decrease in viability with increase in total density and this decrease appears to be frequency dependent, being most noticeable when these lines are at a frequency of 0.9.

As indicated in Table 3.7, viability was lower, except in the case of DF, in Experiment 6 than in Experiment 5. In the conditioned media experiment 300 larvae were placed on media that had already been used to culture 150 larvae, thus a total of 450 larvae were cultured in each vial. In Experiment 6, 500 larvae were cultured in each vial. The observed decreases in viability can hardly be explained by the presence of an extra 50 larvae per vial. Thus it seems that in Experiment 6 the actual presence of all the larvae, exploiting the same resources, at the same time is perhaps a more important element of the competition involved, than the amount of medium available. The amount of medium available per larva would have only been slightly less than in Experiment 5. The same argument would apply if accumulation of detrimental by-products was of major importance. Thus if neither the amount of medium available nor the accumulation of detrimental by-products is the critical factor in these experiments, the conditioning process, which will presumably only alter these two factors, will have little effect on viability. The aim of the final experiment to be described here was to test this hypothesis.

(iv) Experiment 7

(a) Materials and Methods

The viability of five of the six lines was measured in fresh media with the intention of comparing the results with those obtained when viability was measured in homotypically conditioned media (Table 3.3b). Line 4 was omitted due to its poor hatchability and the consequent difficulty involved in collecting larvae of the same age. Fewer replicates were included for some lines for the same reason. 300 first instar larvae of each line tested were transferred to 5cc of cornmeal molasses medium in vials. Vials were maintained at 25°C and all emerging adults were counted.

Comparison of viability in fresh and conditioned media, measured in different experiments might not be valid if there was significant day to day variation in the medium. Therefore a comparison was also included in which viability in fresh and conditioned media was measured using media produced on the same day. For this contemporaneous comparison, line 5 and a sample from the Kaduna population were used. Viability in fresh media was measured by transferring 300 larvae to vials containing 5cc of medium and counting all emerging adults as before. For conditioned media, 150 larvae were transferred to vials containing 5cc of the same batch of media and the viability of 300 larvae of the same line was subsequently measured in this conditioned medium. The results obtained here for line 5 can also be compared with those obtained previously for this line in both fresh and conditioned media and thus provide more direct evidence on day-to-day differences in the medium.

(b) Results

Table 3.8a presents the number of adults emerging from each

Table 3.8

Viability in fresh and conditioned media. Numbers of adults emerging from 300 larvae (a) in fresh media and (b) in contemporaneous comparison of fresh and conditioned media.

(a)

		Line			
	DF	C_3A	3	5	6
	100	160	228	247	232
	94	172	217	222	240
	80	184		239	236
	115	193		242	226
				245	216
				245	225
Mean survival in fresh media	100	177	222	240	229
* Mean survival in conditioned media	114	205	237	259	237
t	1.41	3.39	2.24	3.41	0.97
df	7	7	5	9	9
p	>0.10	<0.025	>0.05	<0.01	>0.20

(b)

	Kaduna		Line 5	
	Fresh media	Conditioned media	Fresh media	Conditioned media
	253	249	253	262
	242	238	256	261
	240	239	236	273
	255	257	231	261
	230	257	227	250
	230	269	254	257
Mean Survival	242	252	243	260
	$t_{10} = 1.52$, $p>0.20$		$t_{10} = 2.78$, $p<0.025$	

* Viabilities in homotypically conditioned media presented in Table 3.3b.

replicate of fresh medium. The mean survival of each line is compared with the mean survival of that line in homotypically conditioned media given in Table 3.3b. In all cases the mean survival in fresh media is lower than the mean survival in conditioned media, and this difference is significant in the case of C_3A ($t_7 = 3.29$; $p < 0.025$) and line 5 ($t_9 = 3.41$; $p < 0.01$).

Table 3.8b presents the results obtained when the same batch of media was used to compare viabilities. Again the mean survival is lower in fresh media, and significantly so in the case of line 5, ($t_{10} = 2.79$; $p < 0.025$). The viability of line 5 given in Table 3.8b when compared with that given in Table 3.8a indicates homogeneity in the media produced from day to day. When viability of this line was first measured in conditioned media (Experiment 5) the mean was 259 $\pm$ 4, compared with a mean of 260 $\pm$ 3 in conditioned media, in the contemporaneous experiment described here. Viability in fresh media, as measured in the first part of this experiment, was 240 $\pm$ 4, compared with 243 $\pm$ 5 in the contemporaneous experiment, which was performed on a different occasion. The comparisons made in Table 3.8a, although of viabilities in media produced at different times, therefore seem valid. A total of six comparisons of viability in fresh and conditioned media are then available and in all cases the conditioning process appears to have had a facilitating effect. In two lines the increased viability is significant at the $2\frac{1}{2}\%$ level and in one line at the 1% level.

III DISCUSSION AND CONCLUSIONS

Kojima and Huang (1972) point to the importance of population density in the establishment of frequency dependent selection in their experiments with Est-6. However what constitutes a low or a high density is not clear. In their 1972 paper Kojima and Huang use six different density levels. They regard densities of 100(50) (i.e. where 50 larvae are used to condition and survival is measured on 100 larvae) and 100(100) as too low to clearly demonstrate any frequency dependent selection. It would appear reasonable to assume that the survival percentages at the six different densities might be a good indication of the level of competition and the strength of any possible selection. The mean survival at the 100(100) density, calculated from Table 1 of their paper, is 85.93% and at the 150(50) density it is 86.26%. It would seem therefore that the 150(50) density might also be regarded as too low to demonstrate frequency dependent selection. However this is the only density level used by Huang et al. (1971) where highly significant frequency dependent selection is reported. Actual survival percentages are not given by Huang et al. (1971) but there appears to be little evidence to indicate that they might be any lower than those observed by Kojima and Huang (1972). The medium used in the earlier experiments was perhaps more nutritious, as yeast, on which the larvae principally feed (Sang, 1949), was added to 5cc Kalmus medium, while no yeast was added to the 5cc cornmeal molasses medium used in the later experiments.

In order to ensure that the density level was sufficiently high for any frequency dependent selection to be apparent, the densities used in the two conditioned media experiments reported here were

higher than those used by Kojima and his associates. The density level in Experiment 4 was 220(150), and in Experiment 5 this was further increased to 300(150). However, even with a high density level and the possibility of many loci contributing to viability differences, none was observed. Frequency dependent selection, resulting in decreased viability in homotypic media relative to viability in heterotypic media, cannot have been an important factor at the loci at which these lines differed. The results of the other two experiments in this section confirm this conclusion and provide possible explanations for the results.

Experiment 6 indicates the importance of factors other than those associated with the medium. The relative viabilities of the lines in mixed culture in this experiment could not have been predicted from their performance in conditioned media. Viability of 500 larvae of these lines in pure culture was not measured. However, as regards supply of nutrients or accumulation of deleterious by-products, (considered by Kojima and Huang (1972) as the key factors in their results) survival in conditioned media, used to culture 450 larvae, can act as a reasonable comparison. Such comparisons clearly indicate that other factors were operating to reduce viability and increase developmental time in C_3A and line 6 in the mixed culture experiment. It is possible that similar results would have been observed with 500 C_3A or line 6 larvae in pure culture. The presence of 500 larvae, irrespective of genotype, competing at the same time for the same resources, may have been sufficient to bring about the changes observed. However, the emergence patterns seem to indicate that direct interference between lines in mixed culture was playing

an important role. Developmental time for each line altered with the frequency of the competing lines and in some cases no flies from the later emerging line were observed at all during the first four or five days of collection. This interference seems unlikely to be the effect of deleterious by-products excreted by the first emerging line, as such effects could also have been observed for these lines in DF and line 3 conditioned media (Table 3.3a). Possibly DF and line 3 interfered with C_3A and line 6, respectively, in such a way as to cause a reduction in feeding rate. The later line may have been inhibited in its efficient utilization of resources while larvae from the competing line were present in the medium, and only when these larvae had left the medium could the later line attain the size necessary for pupation.

Lewontin (1955) and Lewontin and Matsuo (1963) described similar interaction among genotypes in mixed culture. No information is given on emergence patterns but complete reversal of relative predicted fitnesses occurred in two of the five series tested and in no case could performance in mixed culture be predicted from performance in pure culture. All other measures of larval survival in DF and C_3A indicate that C_3A had a much higher survival rate than DF, yet in mixed cultures a complete reversal occurred. No difference in viability between lines 3 and 6 had been previously noted but in mixed culture line 3 had a higher survival rate. Lewontin (1955) concluded that the viability of a genotype is a function of the other genotypes which coexist with it and that such a situation may lead to a stable polymorphism of genotypes. The present results fail to provide evidence of overall frequency dependent selection of the type that

will maintain polymorphism. However, in both C_3A and line 6 lowest viability was observed at highest frequency (Table 3.5). The significantly positive correlation between viability and frequency in DF however, would not result in stable polymorphism. Clearly the relationship between larval fitness and frequency observed in these results cannot be explained by simple frequency dependent selection of the type described by Kojima and his associates. The observed emergence patterns provide further evidence on the complexity of the interaction among lines.

Prolongation of preadult life is a well recognized phenomenon in Drosophila (Sang, 1949). Sokoloff (1955) observed lengthening of developmental time with increased intra-strain competition in D. pseudoobscura and D. persimilis. Dawood and Strickberger (1969a) observed changes in developmental time in mixed culture in D. melanogaster. Developmental time of larvae homozygous for ebony altered significantly with different competitors. With one competitor, the emergence patterns showed almost no overlap and the mean developmental time of ebony was 3 days longer than that observed in pure culture. In the present study, no difference in the developmental times of DF and C_3A in pure culture had been noted. Parsons (1961) concluded that there was a possible correlation between bristle number, fly size and emergence time and that selection for high bristle number might also increase body size and consequently lengthen developmental time. C_3A is the high bristle line and it has a larger body size than DF, the low bristle line. It seems quite probable then that C_3A has a slightly longer developmental time than DF, and under intense competition with DF this difference is greatly exaggerated.

Line 6 had previously been observed to emerge about 12 to 24 hours later than the Kaduna lines. In competition with line 3 a difference of about five days was observed at all frequencies (Table 3.6).

Developmental time in the four lines tended to increase as the frequency of the earlier emerging line increased (Table 3.6). At all frequencies the total density was the same, yet the pattern of changes in developmental time is as if density is increasing and consequently lengthening preadult life. The observed pattern can be explained if the earlier emerging line is completely unaffected by the frequency of the later line and responds only to changes in its own frequency. Changes in the frequency of the earlier line would then be equivalent to changes in density and increased developmental time of that line will result. As the later line appears to be unable to attain the required size for pupation until the earlier line has left the medium, increased developmental time in the earlier line will cause a similar increase in the later line. These changes in developmental time, however, do not bring about an increase in mortality. Dawood and Strickberger (1969a) also observed that increases in developmental time were not necessarily always associated with increases in mortality.

The increase in viability observed in conditioned media relative to fresh media is perhaps surprising if viewed only on the basis of evidence provided by Kojima and his associates on the effects of conditioning. However, facilitating effects similar to those observed here have been observed before. Sang (1949) showed that "larval metabolic products do not impede but may even encourage larval development". Weisbrot (1966) found that both homotypic and heterotypic

media could result in any of the three possible effects, beneficial, detrimental or null. Palabost (1973) found that larvae of the mutant rosy scarlet had higher viability in conditioned media. The results obtained by Dawood and Strickberger (1969b) provide the most interesting comparisons for the present observations. Their experiments were not intended as an investigation of frequency dependent selection, but if such selection were operating through differential utilization of media, it may have been apparent in their results. The density level used, however, was low, consisting of only 80 larvae per vial. Viability of four genotypes was measured in media conditioned by the same four genotypes as well as in control media i.e. fresh media. Two of the four types of conditioned media produced a facilitating effect on all four genotypes, in one case causing a threefold increase in survival over the control level. The other two conditioned media produced both facilitating and detrimental effects. However, if viability in homotypically conditioned media is compared to viability in fresh media (the same comparison as made here in Table 3.8) then it can be seen that in all cases they observed higher survival rates in conditioned media.

Weisbrot (1966) and Dawood and Strickberger (1969b) believed that biotic residues were responsible for the observed alterations in viability. The conditioning process used by these authors was different from the present procedure. A two or three day period of conditioning was used after which the larvae in the media were killed by freezing. In the present experiments the conditioning process lasted for six to seven days until the majority of the larvae

had left the media to pupate. Biotic residues, though possibly different from those involved in the experiments reported by Weisbrot (1966) and Dawood and Strickberger (1969b), may also have played a part in the present results. Another possible explanation for the increased viability observed here in conditioned media may be an increased yeast population. The yeast population in the conditioned media, where it has had six or seven days to develop, is presumably larger than that in the fresh media. As yeast is the primary food source of the larvae (Sang, 1949), an increased population in conditioned media could result in higher survival rates.

The results of tne four experiments presented in this chapter indicate, firstly, that frequency dependent selection, demonstrable through conditioned media, is not a widespread phenomenon. Kojima and Huang (1972) proposed that frequency dependent selection in conditioned media operated tnrougn depletion of nutrients or accumulation of deleterious by-products. In the lines used here no evidence for depletion of nutrients or accumulation of deleterious by-products was found, the conditioning process, if anything, increased survival. Secondly, the results obtained here indicate that direct competition between larvae, competing for the same resources at the same time, is more important than conditioned media in mediating genotypic interactions. Such interactions indicate that the fitness of a particular genotype may be dependent on the other genotypes that coexist with it and that small differences in developmental time between competing genotypes may be of major importance under intense competition.

CHAPTER 4

FREQUENCY DEPENDENT MATING

CHAPTER 4

FREQUENCY DEPENDENT MATING

I INTRODUCTION

The various aspects of sexual selection in Drosophila have been comprehensively reviewed by Petit and Ehrman (1969). The role played by point mutations and inversions in relation to mating success, as well as mating speed and environmental influences, such as light and density, have all been studied in detail. Frequency dependent mating was first reported in D. melanogaster by Petit (1951). Since then it has received much attention from Ehrman (1967, 1969, 1970, 1972), Spiess (1968) and Spiess and Spiess (1969). The phenomenon has been found associated with chromosomal inversions, mutants compared with non-mutants, strains from different geographical areas and the same genotypes raised at different temperatures.

Advantage in mating when rare has generally been associated only with males. When two types of males are present at different frequencies, the rare male has been observed to mate more often than expected. The females in some way recognize the presence of two types of males and show a preference for the rarer type. The cue by which females recognize the presence of two kinds of males is believed to be airborne. Ehrman (1967), using double chambers, separated by a layer of cheesecloth, showed that the advantage of the minority male in the upper chamber disappeared when an excess of these males was present in the lower chamber. Ehrman et al. (1973) isolated a pheromone believed to be involved in mediating this frequency dependent mating.

The aim of the first experiment to be described here was to investigate such frequency dependent mating, involving airborne cues, at ADH, Est-6 and sternopleural bristle loci. The second experiment was intended to investigate the phenomenon at a more general level.

II MATERIALS AND METHODS

(i) Experiment 8

This experiment was similar to the two five generation experiments described in Chapter 2. The two monomorphic lines, DF and C_3A, fixed for the opposite alternatives at the loci under observation, were again used with the segregating Kaduna population. The Kaduna used in this experiment carried no visible markers and DF and C_3A were both homozygous for claret.

Specially designed mating chambers were used for this experiment and one of these is illustrated in Figure 4.1. The inner chamber is separated from the outer chamber by fine mesh nylon, thus mating cannot take place between flies in the inner and outer chambers but airborne cues can pass freely between the two chambers.

All flies were transferred to the mating chamber without etherization. First about 1000 C_3A or DF males, two to four days old, were transferred to the outer chamber. 100 virgin Kaduna females, three to four days old were then placed in the inner chamber. These females were left in the inner chamber for 30 minutes so that they might become aware of the surrounding males. Three to four day old Kaduna males were then added to the inner chamber and mating was allowed to take place over a period of one hour, by which time over 90% of the females had been observed to mate. The inner chamber was then removed and the flies were etherized. The Kaduna males were discarded and 25 females were transferred to each of four food bottles and allowed to lay eggs over a 24 hour period. This density of eggs ensured that there was no overcrowding during the egg to adult stage of the life cycle. Bottles were maintained at

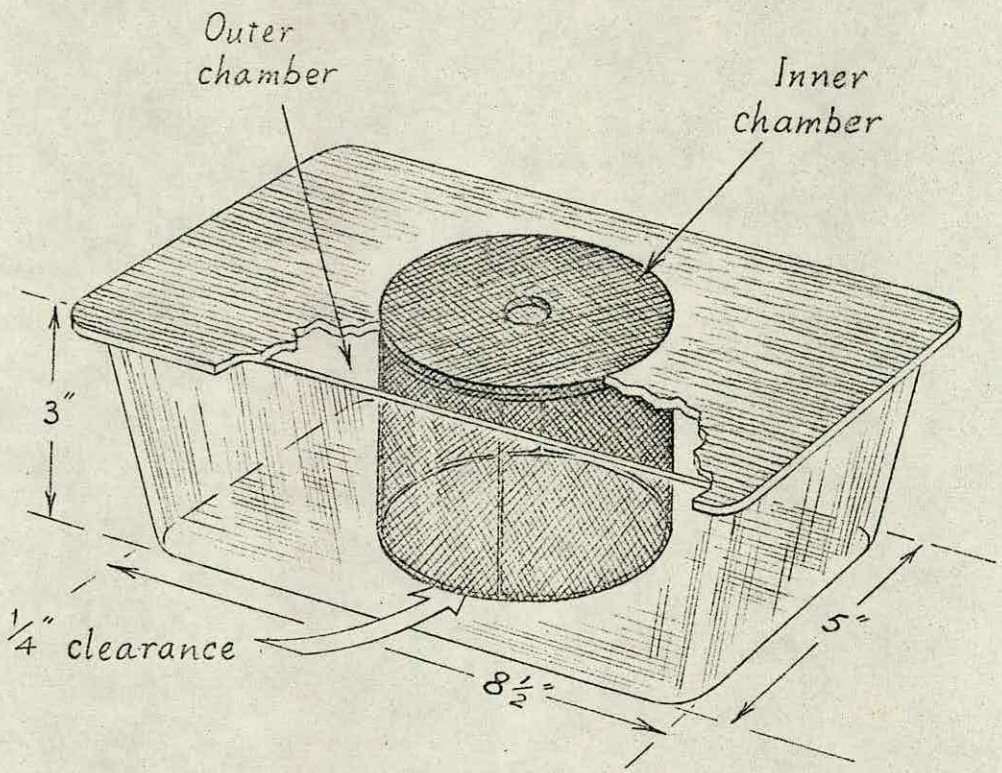

Fig. 4.1. Mating chamber

25°C and on emergence 100 virgin females and males were again collected for the next generation. This procedure was repeated for five generations. The experiment consisted of three replicates where mating took place with DF males in the outer chamber and three replicates with C_3A males in the outer chamber. The gene frequency at both enzyme loci was estimated every generation by electrophoresis of a sample of 48 flies from each replicate. Sternopleural bristle score was recorded in a sample of 30 flies from each replicate at generations 3 and 5.

(ii) Experiment 9

Frequency dependent mating was investigated using different frequencies of caDF and caC_3A. Direct observation of mating, the technique generally utilized in investigations of this nature, was rejected here as both lines have a particularly slow mating speed. Initial investigations showed that when mating took place over a period of 6 hours only 40% of the females had mated. Mating therefore was allowed to take place over a 12 hour period and the usual $\frac{1}{2}$ pint bottles containing medium were used for this purpose.

On emergence sexes were isolated and aged separately for three to four days. As all previous investigations of frequency dependent mating have found little evidence for its occurrence among females, male frequencies only were varied in this study; the frequency of females being kept constant. Each mating bottle then was set up with 25 DF females and 25 C_3A females and a total of 50 males. Five different frequencies of males were studied, 0.1, 0.3, 0.5, 0.7 and 0.9. All flies were transferred to the mating bottles without etherization and mating took place over a period of 12 hours after

which time flies were removed and etherized. Males were discarded and the 25 DF females and C_3A females were transferred singly to 50 vials. Vials were maintained at 25°C and after four days of egg laying the females were discarded.

The genotype of the male by which each female had been inseminated was ascertained by examining the progeny. Two criteria were used in deciding which matings had occurred, bristle score and body colour. The DF line has a mean bristle score of 8 bristles and the females have a large black spot on the 6th abdominal tergite (Rodgers, 1969). The C_3A line has a mean of 49 bristles and the females show an absence of pigment on the 6th tergite. The cross between the two lines has a mean of about 16 bristles and females have the light body colour of C_3A females. Two female offspring from each vial were examined for body colour and bristle number. In the event of there being no female offspring, male offspring were used and the genotype of the male parent was decided on the basis of bristle score alone.

The initial investigation consisted of two replicates of each of the five frequencies and at a later date a further three replicates for each of the two extreme frequencies were set up.

III RESULTS

(i) Experiment 8

If frequency dependent mating is associated with the loci under observation, the predicted changes in the segregating population will be as described for the experiments reported in Chapter 2. In the D replicates (i.e. in Kaduna when DF males are the "competitors" in the outer chamber) the frequency of the fast allele at the enzyme loci and sternopleural bristle score would be expected to increase. The reverse changes would be expected in the C replicates. The results are presented in a similar manner to those in Chapter 2. For the enzyme loci, the standard error of q_F, $\chi^2_{(1)}$ and the E(MS) in the analysis are as described in Chapter 2,(III).

(a) ADH

Figure 4.2a presents the gene frequency at ADH over the five generations. Table 4.1 gives the gene frequency for generation 5 and the analysis of variance for this generation. The mean frequency of the fast allele in the D replicates at the end of the experiment is 0.57 while that in the C replicates is 0.53; this difference, although in the direction expected with frequency dependent selection, is not significant. From Figure 4.2a it can be seen that for the first four generations the D replicates tend to have a lower q_F than the C replicates and generation 5 is the first occasion where the overall mean of the D replicates is higher than the overall mean of the C replicates. There is clearly no evidence for frequency dependent selection in these results.

From Figure 4.2a it is apparent that the gene frequency in the six replicates deviates remarkably little from the equilibrium during

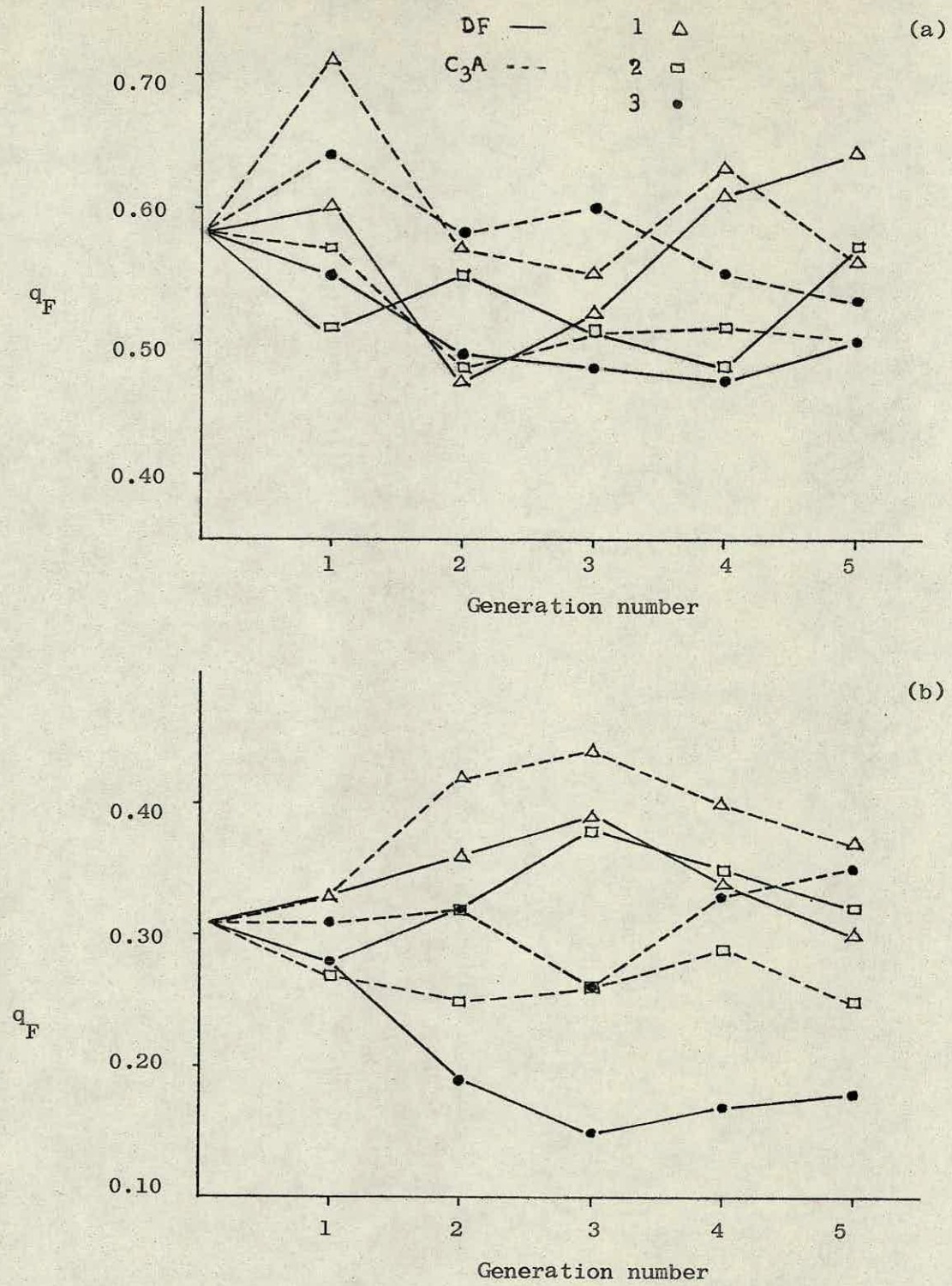

Fig. 4.2. Experiment 8. Gene frequency changes over 5 generations, at ADH(a) and Est-6(b).

Table 4.1

(a) Observed and expected genotype numbers, chi square and gene frequency at ADH at generation 5. Initial gene frequency = 0.58 ± 0.03.

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	17	19	27	22	4	6	2.01	0.64±0.05
2	16	16	23	23	9	9	0.00	0.57±0.05
3	8	12	32	24	8	12	5.33**	0.50±0.05
								$\bar{q}_F$ = 0.57±0.04

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	14	15	26	24	8	9	0.34	0.56±0.05
2	12	12	24	24	12	12	0.00	0.50±0.05
3	13	13	23	23	10	10	0.00	0.53±0.05
								$\bar{q}_F$ = 0.53±0.02

**p 0.025

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.0024	<1.0	
Between replicates Within competitors	4	0.0029	1.12	>0.20
Within replicates		0.0026		

Table 4.2

(a) Observed and expected genotype numbers, chi square and gene frequency at Est-6 at generation 5. Initial gene frequency = 0.31 ± 0.03.

(b) Analysis of Variance.

(a) D Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	5	4	19	20	24	23	-	0.30 ± 0.05
2	5	5	21	21	22	22	0.00	0.32 ± 0.05
3	1	2	15	14	32	33	-	0.18 ± 0.04
								$\bar{q}_F = 0.27 \pm 0.04$

C Replicates

Replicate No.	FF Obs.	FF Exp.	FS Obs.	FS Exp.	SS Obs.	SS Exp.	χ^2_1	q_F
1	5	6	24	21	17	18	0.65	0.37 ± 0.05
2	2	3	17	16	23	24	-	0.25 ± 0.05
3	4	6	26	22	18	20	1.59	0.35 ± 0.05
								$\bar{q}_F = 0.32 \pm 0.04$

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	0.0048	<1.0	
Between replicates Within competitors	4	0.0049	2.24	>0.05
Within replicates		0.0022		

Table 4.3

(a) Mean and standard error of sternopleural bristle score at generations 3 and 5.

(b) Analysis of Variance for generation 5.

(a)

Replicate No.	D replicates Gen. 3	D replicates Gen. 5	C Replicates Gen. 3	C Replicates Gen. 5
1	18.33±0.34	18.90±0.31	18.70±0.34	19.77±0.35
2	18.77±0.27	18.37±0.25	17.47±0.32	18.80±0.28
3	18.70±0.30	19.13±0.31	18.70±0.35	18.60±0.31
Overall mean	18.60±0.12	18.80±0.23	18.29±0.41	19.06±0.36

(b) Analysis of Variance

Source	df	M.S.	F	P
Between competitors	1	2.98	1.07	> 0.50
Between replicates Within competitors	4	8.16	2.94	< 0.05
Within replicates	174	2.78		

the five generations and any tendency to move away from the equilibrium is followed in the next generation by a return. The analysis of variance indicates that at generation 5 there is no significant difference between replicates, any difference being almost entirely accounted for by the within replicate variance.

(b) Est-6

The results for Est-6 are summarized in Table 4.2 and Figure 4.2b. Again there is no evidence for frequency dependent selection, the gene frequency in the D replicates is lower than that in the C replicates. It can be seen from Figure 4.2b that greater fluctuations in gene frequency occur here compared with ADH. At generation 3 the variance between replicates is greatest but replicates with extreme values return towards the equilibrium in the following generations and at generation 5 the variance between replicates, as indicated in the analysis of variance (Table 4.2b), is close to the 5% level of significance.

(c) Sternopleural bristles

Table 4.3 presents the mean and standard error of sternopleural bristle score in the D and C replicates at generation 3 and at generation 5, together with an analysis of variance of bristle score at generation 5. The mean of the C replicates is higher than that of the D replicates at generation 5 but not significantly. The difference between replicates is significant at the 5% level.

(ii) Experiment 9

As both DF and C_3A females mated equally often with both types of males, no distinction is made in the results with respect to females.

Table 4.4a presents the number of successful matings out of a total of 50, at each frequency and the proportion of these achieved by each type of male. At the three intermediate frequencies there seems to be little evidence of any frequency dependence, and DF males appear to mate slightly more often than C_3A males. At the extreme frequencies, however, a fairly consistent pattern of frequency dependence is evident. In all cases where DF males are present at a frequency of 0.10, the frequency of mating is greater than this. In the five replicates where C_3A males are rare only in one case is the frequency of mating less than 0.10. The results given in Table 4.4a, for the last three replicates of each extreme frequency, are those obtained in the second investigation.

In testing the statistical significance of the advantage of the rare male, care must be exercised in combining the results of the initial experiment with those of the three extra replicates, obtained at a later date. These extra replicates were set up when it was clear from the results of the initial investigation that some effect was present at the extreme frequencies. Although the reason for setting up these extra replicates does not alter the results obtained it does affect the null hypothesis and the interpretation of any statistical test performed on the combined results. Chi square tests were therefore performed for each investigation separately as well as for the combined results. Table 4.4b presents the total number of matings, involving each type of male at the extreme frequencies, (i) for the initial investigation, (ii) for the extra replicates and (iii) for the combined results. As there was no significant difference in the mating ability of the

Table 4.4a

Mating success of DF and C_3A males at different frequencies

Freq. of DF	Total no. of matings	Proportion achieved by DF	Proportion achieved by C_3A	Freq. of DF	Total no. of matings	Proportion achieved by DF	Proportion achieved by C_3A
0.1	37	0.24	0.76	0.3	33	0.36	0.63
	37	0.16	0.84		31	0.19	0.81
	39	0.13	0.87				
	36	0.11	0.89	0.5	36	0.78	0.22
	39	0.21	0.79		36	0.50	0.50
0.9	36	0.78	0.22	0.7	33	0.73	0.27
	39	0.92	0.08		40	0.80	0.20
	31	0.87	0.13				
	39	0.90	0.10				
	45	0.87	0.13				

Table 4.4b

Number of matings by DF and C_3A males at the extreme frequencies, for (i) initial investigation, (ii) extra replicates and (iii) combined results

Freq. of DF	(i) DF	(i) C_3A	(ii) DF	(ii) C_3A	(iii) DF	(iii) C_3A
0.1	15	59	17	97	32	156
0.9	64	11	101	14	165	25
χ^2_2	9.96		3.58		12.40	
P	< 0.01		> 0.10		< 0.005	

two types of males at the extreme frequencies, the expectations are based on the proportions of each type of male present. The chi square, with two degrees of freedom, for (i) is significant at the 1% level. Clearly the rare male has an advantage, and this advantage is again apparent in the extra replicates although in this case it is not significant. Combining the results from the two investigations yields a chi square = 12.4 indicating an overall significant advantage for the rare male. In some replicates this advantage was particularly marked, with as many as 8 or 9 of the total number of matings being achieved by the 5 rare males present.

IV DISCUSSION AND CONCLUSIONS

The results presented here for the investigation of frequency dependent mating at the three specific loci are in agreement with those obtained for Experiment 2, Chapter 2, summarized in Tables 2.4-2.6 and Figure 2.2. In the previous experiment direct competition between Kaduna and the two monomorphic lines took place during mating and egg to adult development. No evidence for frequency dependent selection was found at any of the three loci. In this experiment largernumbers of flies per replicate were used in an effort to reduce the drift variance and mating only was investigated.

It is possible that the indirect form of competition used here was not sufficient to mediate frequency dependent mating. However, considerable experimental evidence has been amassed to indicate that the females recognize the presence of two types of males by means of airborne cues. Ehrman (1970) artificially induced minority advantage in mating, using a double chamber, when there was no difference in the actual frequencies of the competing males. Ehrman (1972) also described experiments where crushed individuals or extracts from dead flies were used to demonstrate frequency dependent mating. Bennet-Clarke and Ewing (1970) stressed the importance of auditory cues. Either type of cue could have been perceived by Kaduna females in the present experiment. When DF males were in the outer chamber, the Kaduna females were receiving cues only from flies which were homozygous slow at both ADH and Est-6 and had low bristle scores. Those Kaduna males carrying the fast enzyme alleles and high bristle alleles would then have been rare, and if such rarity conferred

an advantage an increase in the frequency of these alleles would have been expected to occur during the five generations of the experiment. The reverse situation would be expected when C_3A males were present in the outer chamber. The results indicate no such changes.

If frequency dependent selection then was not operating at these loci it can again be asked, as in Chaper 2(IV), is the observed variance among replicates the result of random drift or has some other form of selection played a part? The analysis of variance of 5th generation results (Table 4.1) indicates that for ADH there was no significant variance between replicates. However, from Figure 4.2a it can be seen that such a conclusion may perhaps be slighly misleading. If the gene frequencies in each replicate at generations 4 and 5 are considered together, the variance between replicates is just significant at the 5% level. Even though the 5th generation's results may give an underestimate of the true variance between replicates for ADH, comparison of the gene frequency changes over the five generations at the two enzyme loci (Figure 4.2) indicates that the gene frequencies at ADH tended to remain closer to the equilibrium value throughout.

Calculating the values of F and N, under the hypothesis that drift alone is responsible for the observed variance, as in Table 2.11, yields the following results for the three loci.

	F	N
ADH	0.0008	3045
Est-6	0.013	195
Bristles	0.048	50

Although these F values are far too small to enable any satisfactory distinctions to be made between drift and selection (Nicholas, 1974), the heterogeneity between them is very marked. An estimate of N of 3045, when there were only 200 flies per replicate, seems to indicate that some form of stabilizing selection is indeed operating at ADH, even if allowances are made for the possible overestimate given by using generation 5 results alone. In general these results agree with those obtained in Chapter 2, indicating the presence of some form of selection tending to maintain the equilibrium value at ADH but providing no evidence for selection at either Est-6 or the bristle loci.

The significant advantage of the rare male in mating, found in the second experiment here, is not necessarily contradictory to the results obtained in the first experiment. One possibility is that factors other than airborne cues were involved in Experiment 9, although there is little evidence for the existence of such factors. It seems more likely that loci other than ADH, Est-6 and bristle loci were involved in Experiment 9. DF and C_3A presumably differ at many other loci besides these. The generations of selection for sternopleural bristle number in these lines have resulted in differences in body size and body colour. The experiments reported in Chapter 3 indicate differences in larval viability and developmental time at high densities. Thus differences at any of numerous loci may have been responsible for the rare male advantage observed. If such advantage is generally mediated by some type of pheromones, as indicated by Ehrman *et al.* (1973), then these pheromones appear to be affected by alterations at many loci as well

as by environmental changes. Frequency dependent mating, as previously mentioned, has been found associated with several point mutations, inversions and identical genotypes raised at different temperatures. From the evidence provided by Experiment 8 here, ADH, Est-6 and sternopleural bristle loci are not among the loci capable of affecting such pheromones, but DF and C_3A presumably differ at one or more of the loci involved.

CHAPTER 5

GENERAL DISCUSSION

CHAPTER 5

GENERAL DISCUSSION

Since Lewontin and Hubby (1966) produced evidence of vast amounts of enzyme and protein variation in natural populations, much of population genetics has been devoted to an effort to explain their findings. While selectionists strive to find new ways of convincingly demonstrating the part played by selection, neutralists find new theories to explain away their observations.

Similarity of allelic frequencies in different populations (Praskash et al. 1969, Ayala et al. 1971) has been considered as strong evidence for selection. However, Kimura and Ohta (1971) and Kimura and Maruyama (1971) have shown that such observations can be equally well explained by the neutralist hypothesis.

Cavalli-Sforza (1966) proposed the use of the standardized variance of gene frequency, F, to distinguish between drift and selection. Lewontin and Krakauer (1973), using this method, demonstrated that, in human populations, the variation in F values among loci was larger than could be explained by drift alone, indicating that some loci must be under selection. However, advocates of the neutralist hypothesis do not deny that some loci are subject to selection. Yamazaki and Maruyama (1974), using a statistic that is independent of population structure, showed that data on enzyme polymorphism fitted the neutralist hypothesis whereas data on blood group polymorphism led to the conclusion that balancing selection was involved.

Findings of linkage disequilibrium, as proposed by Franklin and Lewontin (1970), would perhaps provide more definite evidence for

selection. Praskash and Lewontin (1968 and 1971) found correlations between enzyme loci and inversions on the same chromosome in D. pseudoobscura and D. persimilis. Charlesworth and Charlesworth (1973) found linkage in populations of D. melanogaster. However, linkage disequilibrium of the strength suggested by Franklin and Lewontin (1970) remains to be demonstrated.

Efforts to find selection associated with single loci seem fraught with difficulties. First the fitness estimation problems, as pointed out by Prout (1965, 1969 and 1971) are numerous. Secondly selective coefficients may be too small to demonstrate clearly in laboratory experiments. Selective coefficients of 0.01 will, in most natural populations, be sufficient to stem the loss of variation through drift. Demonstration of selective coefficients of this order would require lengthy laboratory experiments. Furthermore, if particular alleles are only advantageous in heterogeneous environments it may not be possible to detect selection in a constant laboratory environment. However, Day et al. (1974) proposed that detection of the extent to which alleles at polymorphic loci differ biochemically would help in discriminating between selection and drift at single loci. Nevertheless, even if it is possible to demonstrate convincingly the presence of selection at one or two loci, this says nothing for the widespread occurrence of that particular mode of selection or for selection in general. In fact it seems that the exceptional cases will be easiest to find, sickle celled anaemia being a classical example.

Kojima and Yarbrough (1967) proposed frequency dependent selection as a general mechanism for the maintenance of enzyme polymorphism.

However the generality of the mechanism is far from certain. The findings of Kojima and his associates, of frequency dependent selection at ADH and Est-6, constitute the only reported cases of frequency dependent selection at electrophoretically detectable loci. Closer examination of their results shows that these may well be exceptional cases that in no way indicate the generality of the mechanism. The changes in gene frequency observed at ADH (Kojima and Tobari, 1969b) and Est-6 (Kojima and Yarbrough, 1967) are indicative of extremely large selective coefficients which even the most "naive panselectionist" could not consider as widespread. Selective coefficients ranging from about 40% to over 90% were observed by these authors at ADH, when the gene frequency was perturbed from its equilibrium value of $q_F = 0.60$. This intense selection is all the more remarkable in view of the fact that two of the four cages set up from the original population were fixed for the fast allele and in the third cage the frequency of fast was 0.90. It is not clear why an equilibrium with the fast allele at a frequency of 0.60 was so strongly selected for in the cage which provided the experimental material. Such considerations seem to indicate that the frequency dependent selection reported was not even a general phenomenon in these four cages which originated from the same population.

Further indications of the non generality of such frequency dependent selection are provided by Yamazaki (1971) who tested for frequency dependent selection at Est-5 in D. pseudoobscura in carefully designed experiments, but found no evidence for its occurrence. McIntyre and Wright (1966) found that the Est-6 locus in D. melanogaster could be neutral if care was taken to avoid the effects of linkage.

Professor A. Robertson (personal communication) set up cage populations some 6 years ago with ADH at non equilibrium gene frequencies. No significant return towards the equilibrium has as yet been observed. In experiments specifically designed to test for frequency dependent selection at ADH, similar to those reported in Chapter 2 here, Robertson found no evidence for frequency dependent selection over 18 generations. As negative results often remain unpublished, these examples are quite possibly only a small proportion of the total.

The present study includes experiments designed to detect frequency dependent selection during the egg to adult stage of the life cycle, as found by Kojima. Even though these experiments were capable of detecting far weaker selection than that observed by Kojima, none was apparent at either ADH, Est-6 or associated with sternopleural bristle loci. Selective coefficients greater than about 7% would have yielded statistically significant results in Experiments 1, 2 and 3. However, not even the slightest trace of any frequency dependent selection was found. As previously mentioned these experiments were not such as to rule out all selection and can not be regarded as evidence in favour of the neutralist hypothesis. When the results of Experiment 8 (Figure 4.2) are considered in conjunction with those of Experiments 1 and 2, it seems possible that some form of selection may have been operating to maintain the equilibrium gene frequency at ADH. No definite evidence of any selection associated with Est-6 or sternopleural bristles was apparent.

Kojima believed that frequency dependent selection associated with larval survival was a widespread phenomenon and he proposed that it might operate through each genotype occupying a separate ecological niche in the environment. It is difficult to imagine so many separate

ecological niches in the one environment. However, Huang et al. (1971) and Kojima and Huang (1972) used conditioned media to demonstrate the existence of separate ecological niches associated with Est-6. They reported reduced viability in homotypically conditioned media relative to heterotypically conditioned media. They believed that a particular genotype used to condition the media utilized certain nutrients or left behind deleterious by-products, thus reducing the chance of survival of the same genotype in that conditioned media.

In Experiments 4 and 5 of the present study lines differing at many loci were used to investigate viability in homotypic media relative to heterotypic media. Density levels higher than those of Kojima were utilized but no evidence of viability differences were found. Furthermore, the mechanisms through which Kojima believed the conditioning process to operate were completely absent. The results of Experiment 7 indicate that such conditioning if anything enhances survival, a phenomenon previously observed by Dawood and Strickberger (1966b).

The results of Experiment 6 indicate that the presence of larvae exploiting the same resources at the same time is a more important factor than conditioned media in mediating interactions between genotypes. Both the viability and developmental time of the lines used in Experiment 6 could not have been predicted from their performance in conditioned media. This experiment utilized direct competition between lines present at different frequencies and was the only occasion where any indication of frequency dependent selection operating on larval survival, was found. However, in the one case where the frequency dependence was statistically significant it was not

of the type capable of maintaining polymorphism - increases in fitness being observed with increases in frequency. The complicated developmental patterns observed in this experiment were far more striking and seem to have been perhaps a more important factor in mediating the genotypic interactions found.

Apart from the work of Kojima and his associates, various other forms of frequency dependent selection have been proposed in the literature. Haldane (1949) speaking of disease resistance pointed out that "...it is an advantage to the individual to possess a rare biochemical phenotype. For just because of its rarity it will be resistant to diseases which attack the majority of its fellows." Self sterility alleles in higher plants provide another example of frequency dependent selection. Possibly the best understood and most widespread occurrence of such selection is in predator prey situations where it has been associated with polymorphism in both the prey (Clarke, 1969) and the predator (Paulson, 1973).

In Drosophila frequency dependent selection appears to be a fairly well established phenomenon in mating. However, frequency dependent mating associated with specific enzyme loci has not been reported. In the present study, Experiment 8, provided no evidence for its occurrence at ADH or Est-6. This experiment also indicated that such selection was not a factor in the maintenance of variation at sternopleural bristle loci. Frequency dependent mating, of a possible multigenic origin, was found in Experiment 9. It would be of interest to further investigate the phenomenon in the two highly selected lines utilized. Could this frequency dependent mating be demonstrated with the mating cages used in Experiment 8? If so, could it be established that DF and C_3A do in fact produce different

pheromones? Ehrman et al. (1973) believed that frequency dependent mating in Drosophila was mediated through pheromones. Although the advantage in mating possessed by the rare male has been well established in laboratory experiments, its general occurrence in natural populations is not easy to envisage.

Kojima (1971) criticised the use of hypotheses which assumed constant selective values. He maintained that such models gained popularity, not because they could be shown to apply to many biological situations, but because of their simplicity and traditional use. His alternative hypothesis of frequency dependent selection is now beginning to gain popularity in population genetics literature as an explanation for protein polymorphism. The reasons for its popularity appear to be no more convincing. It is also mathematically simple to handle and has the advantage of overcoming the problem of the segregational load present in the heterotic model. Also because of its occurrence in predator prey relationships and in mating in Drosophila, it is assumed that it occurs at enzyme and other protein loci. There appears to be little justification for such an assumption. Furthermore, the results of Kojima and his associates cannot be regarded as being indicative of the generality of the mechanism at enzyme loci. Some internal inconsistencies are present in their results and the extremely intense selection observed seems to indicate the presence of other unexplained factors which could well render their results unreliable. In the present study evidence for frequency dependent mating has been found but frequency dependent selection was completely absent at the enzyme loci studied and also at sternopleural bristle loci. The results presented are in direct contradiction to

those of Kojima as regards the mode of selection at Est-6 and ADH and the effects of conditioned media. It would appear then that the evidence available at present provides little justification for regarding frequency dependent selection as an established mechanism for maintaining protein polymorphism.

SUMMARY

A search for frequency dependent selection in Drosophila melanogaster was undertaken. Experiments, designed to detect such selection associated with the maintenance of genetic variation at alcohol dehydrogenase, esterase-6 and sternopleural bristle loci, were performed. Egg to adult viability and mating were both investigated, but at neither stage of the life cycle was there any evidence of frequency dependent selection associated with the three systems under observation.

Conditioned media was used to investigate frequency dependent selection of a multigenic origin in highly selected lines and in inbred lines. No reduction in larval viability was observed in homotypically conditioned media relative to viability in heterotypically conditioned media. Further investigations comparing viability in fresh and conditioned media indicated that the conditioning process had, in fact, a facilitating effect in all the lines tested.

Direct larval competition indicated that the presence of larvae competing for the same resources at the same time was more effective than the use of conditioned media in demonstrating genotypic interactions. In all cases performance in mixed culture could not have been predicted from performance in conditioned media, a complete reversal in relative predicted fitnesses occurring in one case. Some indication of frequency dependence was observed but these results were confounded with complicated developmental patterns which appeared to be more important than any frequency dependence in mediating the genotypic interactions observed.

Frequency dependent mating was also investigated using highly selected lines. The results indicated that the rare male had a significant advantage in mating but it was not possible to associate this advantage with any specific loci.

The results are discussed in relation to the relevant work in the field. A critical examination of the literature, associating frequency dependent selection with the maintenance of protein polymorphism, is made. It is concluded that, on the basis of the evidence at present available, there is little justification for regarding frequency dependent selection as an established explanation for protein polymorphism.

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